1970

Germination-dormancy and promoter-inhibitor relationships in Setaria lutescens seeds

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GERMINATION-DORMANCY AND PROMOTER-INHIBITOR RELATIONSHIPS IN *SETARIA LUTESCENS* SEEDS

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Physiology

Approved:

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1970

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INTRODUCTION

Seeds of yellow foxtail (Setaria lutescens) exhibit an annual periodicity of germination and dormancy, characteristic of many annual weeds in temperate zones. Seed dormancy is the survival mechanism which prevents the fall germination of species which are not winter hardy. In most species including yellow foxtail, the winter exposure of seeds to cool moist conditions terminates dormancy. Improved methods for the control of annual weeds may depend on a better understanding of the sequence of physiological events which power this biological time clock.

There is increasing evidence that germination promoters and inhibitors are involved in the regulation of seed germination and dormancy. This evidence comes from studies which have examined the influence of exogenous regulators on seed germination, and from studies in which endogenous regulators were extracted and characterized. It appears that plant hormones such as abscisic acid, the gibberellins, and the cytokinins interact in the regulation of seed germination.

This study is an attempt to characterize the regulatory mechanism of seed germination and dormancy in yellow foxtail, and to examine the proposal that germination promoters and inhibitors are instrumental in that regulation. Two approaches were utilized. First, the influence of plant hormones such as abscisic acid, the gibberellins, and the

cytokinins on seed germination was examined by germination tests with exogenous test solutions. Secondly, attempts were made to extract endogenous germination regulators, and to correlate their levels with the observed state of dormancy.

LITERATURE REVIEW

Dormancy is a period in the life cycle of a plant or plant part in which active growth is suspended temporarily. Seed dormancy is a special adaptation to survival under adverse environmental conditions. In the temperate zone seed dormancy in annual plants synchronizes the initial phase of plant growth and development (germination) with the beginning of the growing season. Dormancy prevents germination immediately following seed maturation in the fall of the year near the end of the growing season, thus insuring seed survival until spring when it can germinate and develop into a seedling at the beginning of the growing season.

Recent review articles on seed dormancy by Amen (1968), Kelly (1969), and Wareing (1969) indicate that there is increasing evidence that dormancy is under the control of endogenous germination promoters and inhibitors. Two types of investigations lend support to the theory that dormancy is under the control of plant hormones--the study of the effects of exogenous hormones, and the study of endogenous hormone levels.

Seed germination in many instances can be controlled or influenced by exogenously added plant hormones. In a wide variety of plant species gibberellins can promote germination. Abscisic acid has been shown to be a potent

and general inhibitor of seed germination. In some species cytokinins can reverse the inhibition of abscisic acid.

In several investigations changes in levels of endogenous promoters and inhibitors during emergence from dormancy are well correlated with the state of dormancy of the seed. It has been shown in one species of seed that during stratification, which terminates dormancy, the potential to produce gibberellin is increased, and in other species it has been shown that the level of natural inhibitor is decreased by stratification.

Stratification, which is the exposure of moist seeds to low temperature, is the natural process of dormancy termination in many species including yellow foxtail. In the natural environment yellow foxtail seeds mature in the fall and then are exposed to the moisture of the soil and the cool temperatures of fall and winter. These conditions result in the transformation of innately dormant seeds in the fall to seeds in a state of enforced dormancy in the winter. Natural stratification can be successfully mimicked in the laboratory.

Abscisic Acid Inhibition

Abscisic acid is a potent and evidently a general inhibitor of seed germination in a wide range of plant species as indicated in Table 1. Inhibition generally occurs at concentration between 1×10^{-6} and 1×10^{-4} M.

Table 1. Literature reports of seed germination inhibition by abscisic acid

Investigator(s)	Year	Species	Concentration used (x 10^{-6} M)
Sondlheimer and Galson	1966	ash	10
Khan	1967	lettuce	200
Sumner and Lyon	1967	1 annual and 3 perennial grasses	3-15
Bradbeer	1968	hazel	30
Khan	1968	lettuce	
Sankhla and Sankhla	1968a	lettuce	1-15
Sankhla and Sankhla	1968b	2 desert species and cress	15 150 0.4-0.8
Gabr and Guttridge	1968	cress	-
Rudnicki	1969	apple embryos	0.1-12

In apple embryos Rudnicki (1969) found that increasing periods of stratification decreased the sensitivity of the embryos to abscisic acid inhibition. Embryos stratified for longer periods of time required a larger quantity of abscisic acid to inhibit germination.

A review article by Addicott and Lyon (1969) on the physiology of abscisic acid indicates that the effects of abscisic acid on seed germination are transient and the

hormone is evidently completely nonphytotoxic. The inhibition is relieved when abscisic acid is removed from the seed.

Gibberellin Promotion

The involvement of gibberellins in the germination process is well documented. The role of gibberellin in the germination of barley seed is probably the best known of all plant hormone responses. Chrispells and Varner (1967a) have demonstrated that the presence of gibberellin is required to stimulate the synthesis of hydrolytic enzymes in the aleurone layer. These enzymes then break down stored carbohydrates in the endosperm of the germinating barley seed which are utilized by the developing seedling.

As summarized by Junttila (1970, p. 425), "Amen (1968) suggested that gibberellin is probably a universal component of an inhibitor-promoter complex which constitutes the naturally occurring mechanism in the regulation of seed dormancy and germination." Khan and Waters (1969) proposed a scheme for the hormonal control of seed dormancy and germination in which they suggested that the role of gibberellin is to provide the primary stimulus for germination.

Exogenously added gibberellins have been shown to stimulate germination or break seed dormancy in a wide range of species. Khan, Goss, and Smith (1957), Evenari et al. (1958), and Khan (1960) have shown that treatment

with gibberellic acid resulted in the dark germination of light requiring lettuce seeds. Frankland (1961) demonstrated that gibberellin could be substituted for stratification in Corylus avellana to break dormancy. Trelawny and Ballantyne (1963) and Tager and Clarke (1961) found that seeds which normally require alternating temperatures would germinate under constant temperature if gibberellin was supplied. Many other investigators including Kallio and Piroinen (1959), Corns (1960), Bradbeer (1968), Khan and Waters (1969), Thompson (1969), and Junttila (1970) have demonstrated a germination response to gibberellin.

However, not all species respond to exogenous gibberellin treatment. Kallio and Piroinen (1959) reported that only 8 of 50 species tested responded to gibberellin and Corns (1960) demonstrated that only a few of the 19 weed species tested responded to gibberellin treatment. Lack of response to gibberellin does not preclude sensitivity to the hormone. There is considerable evidence which indicates that fruit and seed coats are not readily permeable to gibberellin. Kallio and Piroinen (1959) have shown that scarification of the seed coats of Trolluis europaeus and Geranium sylvaticum with sulfuric acid greatly increased the response to gibberellin. Tager and Clarke (1961) found that gibberellin could replace the requirement for fluctuating temperatures in Asclepias fruticosa only if the seed

had been previously pricked with a needle. Frankland (1961) found that removal of the pericarp and testa was required to obtain a gibberellin response in Corylus avellana and Fagus sylvatica. Evidently, the entry of gibberellin into the seed is facilitated by damage to the fruit or seed coat. The wide range of germination responses to gibberellin may simply reflect differences in fruit and seed coat permeability to the hormone.

Gibberellic acid (GA_3) is the most readily available, and hence the most commonly used gibberellin. However, there is evidence that other gibberellins, primarily GA_4, are far more active in seed germination. Ikuma and Thimann (1963) found that gibberellin D, which is a combination of GA_4 and GA_7, was 100 times more effective than GA_3 in promoting the germination of lettuce seeds in the dark. Thompson (1968) reported that GA_4 was much more effective than GA_1, GA_3, GA_5, GA_7, and GA_9 in promoting dark germination of strawberry seeds. Brian, Hemming, and Lowe (1962) found that GA_4 and GA_7 were the most active in stimulating the dark germination of Grand Rapids lettuce seed of the nine gibberellins examined. Hashimoto and Yamaki (1959) demonstrated that GA_4 was considerably more effective in promoting dark tobacco seed germination than GA_1, GA_2, or GA_3.

Promoter-Inhibitor Interactions

Although abscisic acid is a potent inhibitor of seed germination, in some instances this effect can be overcome by interaction with other plant hormones. There are two considerations to be made for promoter-inhibitor interactions; first, does the inhibitor block promotion mediated by the promoter, and second, does the promoter block inhibition caused by the inhibitor. Specifically for seed germination, considering abscisic acid as an inhibitor and gibberellin and cytokinins as promoters, three generalizations can be made. Abscisic acid can block gibberellin-mediated germination promotion. Gibberellin usually is not effective in reversing abscisic acid inhibition. Cytokinins are often effective in neutralizing the inhibitory effects of abscisic acid.

Based on several years of research, in which the above generalizations became apparent, Khan and Waters (1969) have developed as a working hypothesis a scheme which suggests roles for three hormones in the control of seed germination and dormancy. In this scheme gibberellin or related compounds serve as the primary stimulus for germination. Thus, gibberellin is required to initiate the series of reactions which result in seed germination. The endogenous inhibitor, which is probably abscisic acid, has the potential to block the gibberellin-mediated germination. According to

the proposal the cytokinins function by antagonizing specifically the activity of the endogenous inhibitor and thereby allowing gibberellin to initiate germination. Thus, even though an inhibitor was present, it would be unable to function due to the neutralizing action of the cytokinin present. According to this scheme the presence or absence of these three hormones will determine whether the seed germinates or remains dormant.

There is considerable evidence which supports the above described working hypothesis, and little evidence which contradicts it. Khan (1967a) and Khan (1967b) have shown the interaction between exogenous promoters and inhibitors in lettuce seed germination. Abscisic acid inhibits the germination of these seeds both in the light and dark. This inhibition was overcome by the two cytokinins, kinetin and zeatin of which the latter was most effective. Very similar results were obtained by Sankhla and Sankhla (1968a). They found also that the inhibition of lettuce seed germination caused by abscisic acid could be reversed completely by kinetin. Gibberellin and indole acetic acid were ineffective in this role.

Khan (1968) demonstrated that the gibberellic acid induced dark germination of lettuce seed could be inhibited by abscisic acid, and that this inhibition could be reversed by kinetin or benzyl-adenine. In the two previously cited

investigations, interactions between two hormones were considered; however, in this investigation three hormones were involved in the interactions. The interactions between the three hormones in this system are very much in accord with the hypothesis proposed by Khan and Waters (1969).

Recent work by Sankhla and Sankhla (1968b) has shown similar interactions for other species. The effect of promoters and inhibitors on the germination of three species was investigated. It was found that abscisic acid inhibited the germination of all three species. Kinetin and zeatin were able to completely reverse abscisic acid inhibition of germination for Leptandania pyrotechnica, partially reverse the inhibition for Arabidopsis thaliana, and the two cytokinins had no effect on abscisic acid caused germination inhibition for Ipomoea pentaphylla. Indole acetic acid and gibberellin were ineffective in reversing abscisic acid inhibition of germination.

In intact barley seeds, abscisic acid inhibits the production of α-amylase. Khan and Dowing (1968) have shown that kinetin and benzyl-adenine are more than twice as effective as gibberellic acid in reversing this inhibition.

In opposition to the above generalizations are the results of Sondlheimer and Galson (1966). They found the inhibition of germination in excised ash embryos could be almost completely reversed by gibberellic acid, but kinetin

had little effect.

Endogenous Promoter-Inhibitor Levels in Seeds with a Stratification Requirement

The dormant seeds of many species require a period of stratification to overcome dormancy. This requirement for germination is met in the natural environment by overwintering in moist soil. Obviously during the transformation of a dormant seed to a nondormant seed, which occurs during stratification, one or more physiological changes occur. On the basis of the promoter-inhibitor theory of seed dormancy there are several possibilities which could explain the shift in the physiological state of the seed. A decrease in the level of inhibitor, or an increase in the level of promoter, or both may occur during the termination of dormancy by stratification.

There is recent and strong evidence that the concentration of abscisic acid decreased during stratification in some species. There is also evidence that during the process of stratification seeds of some species develop the potential to produce gibberellin; however, the gibberellin is evidently not produced at the low stratification temperature but is subsequently produced at the higher temperature required for germination.

In an attempt to correlate the inhibitor content with the physiological state of the seed, several investigators

have examined changes in the concentration of germination inhibitors as a function of stratification time. After studying dormancy in ash seeds, Villiers and Wareing (1965) concluded that the activity of inhibitors was not decreased in either embryo or endosperm during low-temperature after-ripening. However, by employing new techniques and recently obtained knowledge concerning abscisic acid inhibition Sondlheimer, Tzou, and Galson (1968) obtained different results. Using Milborrow's quantitative "racemate dilution" method of determining abscisic acid concentration they found the inhibitor present in the seed and the pericarp. During the stratification treatment, which releases dormancy in ash seeds, the abscisic acid levels decreased by 37 percent in the pericarp and 68 percent in the seed. They concluded that the abscisic acid present in the pericarp was not involved in the regulation of dormancy; however, the abscisic acid in the seed apparently does play a regulatory role in germination.

Several investigators have examined inhibitor content of seeds by chromatographing the acid fraction of the seed extract, and then subjecting portions of the developed chromatograph to bioassay. Inhibitor bands detected by this technique are then compared to abscisic acid standards. This method of comparing seed extract components to standards is often referred to as co-chromatography. By employing

these techniques Lipe and Crane (1966) found an inhibitor in dormant peach seeds which disappeared when the seeds were exposed to stratification. Ten to 12 weeks of stratification were required to break dormancy; however, the investigation indicated that the inhibitor disappeared by the 6th week of stratification. Co-chromatography and ultraviolet absorption spectra indicated that the natural inhibitor was the same as or very similar to abscisic acid.

Also by chromatography and bioassay Martin et al. (1969) and Rudnicki (1969) found very similar results for walnut and apple seeds respectively. A natural inhibitor was present which disappeared during a period of stratification. In both cases the disappearance of the inhibitor occurred before the termination of dormancy by several weeks. The natural inhibitor appeared to be abscisic acid on the basis of co-chromatography and ultraviolet absorption spectra. Abscisic acid reinstated dormancy in stratified apple and walnut seeds.

A substantial amount of investigation has been conducted on the effect of stratifying conditions on the concentration of gibberellins in hazel (Corylus avellana L.) seeds. Frankland and Wareing (1966) reported that stratification, which is required to terminate dormancy of hazel seeds, resulted in an increase in the gibberellin content of the seeds. However, the resulting concentrations of gibberellin

appeared to be much too low to account for the germination of the stratified seeds.

Bradbeer (1968) and Ross and Bradbeer (1968) found that exposure to germination temperature (20 C) following the stratification period (5 C) increased the gibberellin concentration approximately 70 times. These results suggest that the role of stratification may be to prepare the seed for gibberellin synthesis, which subsequently occurs at the germination temperature.

Further support for this proposal comes from the work of these investigators employing the use of the gibberellin synthesis inhibitor chlorocholine chloride (CCC). They found that CCC had no effect during the stratification period, but did inhibit germination when supplied during the germination period following stratification. This indicates that the period sensitive to gibberellin synthesis inhibition is the actual germination process rather than the prior stratification period.

Thus, there is strong evidence that in ash, apple, peach, and walnut seeds the natural inhibitor decreases in concentration during stratification; in hazel seeds, stratification develops or activates the gibberellin producing system which subsequently becomes productive at higher germination temperatures. It remains unclear if both these processes occur in the same species, or if the

dormancy termination resulting from stratification occurs by different mechanisms in different species.

There is evidence that dormancy in several species of the grass family is caused by natural germination inhibitors. Research by Naylor and Simpson (1961) on Avena fatua indicated that the control of germination during the period of after-ripening was through changes in the inhibitor content. An investigation of wheat seed germination by Miyamoto, Tolbert, and Everson (1961) indicated that dormancy was due to endogenous inhibitors, and that the inhibitors were located in the seed coat.

Dey and Sircar (1968) found a naturally occurring inhibitor present in dormant rice seeds, but absent in nondormant seeds. The inhibitor was detected in the rice husk and embryo, but not in the endosperm. The endogenous germination inhibitor was compared with abscisic acid in two paper chromatography solvent systems and two bioassays and found to be very similar.

Wurzburger and Lesheml (1969) extracted a germination inhibitor from the husk of Aegilops kolchyi a grass species that grows in Israel. The response to the inhibitor was very similar to abscisic acid in four different bioassays. No inhibitor was found in one year old glumes.

Seed Dormancy in Yellow Foxtail

Several investigators have studied seed dormancy in yellow foxtail. Nieto-Hatem (1963) found two types of dormancy in this species, one caused by the caryopsis, the other by the hull. For seeds in which the dormancy was caused by the hull, removal of the lemma and palea (hull) terminated dormancy. For seeds which were in a state of caryopsis dormancy removal of the hull had no effect; however, dormancy in these seeds could be terminated by stratification. Embryos excised from either dormant or nondormant seeds grew normally on nutrient medium, suggesting that embryo dormancy was not a factor in the dormancy of yellow foxtail seeds.

Nieto-Hatem found no difference in the rates of water uptake between dormant and nondormant seeds. Attempts to characterize dormancy on the basis of known organic acid, fatty acid, and carbohydrate constituents were not successful. No important differences in these compounds were found between dormant and nondormant seeds. Leaching in water improved the germination of seeds which indicated that a water soluble inhibitor may be involved in the dormancy.

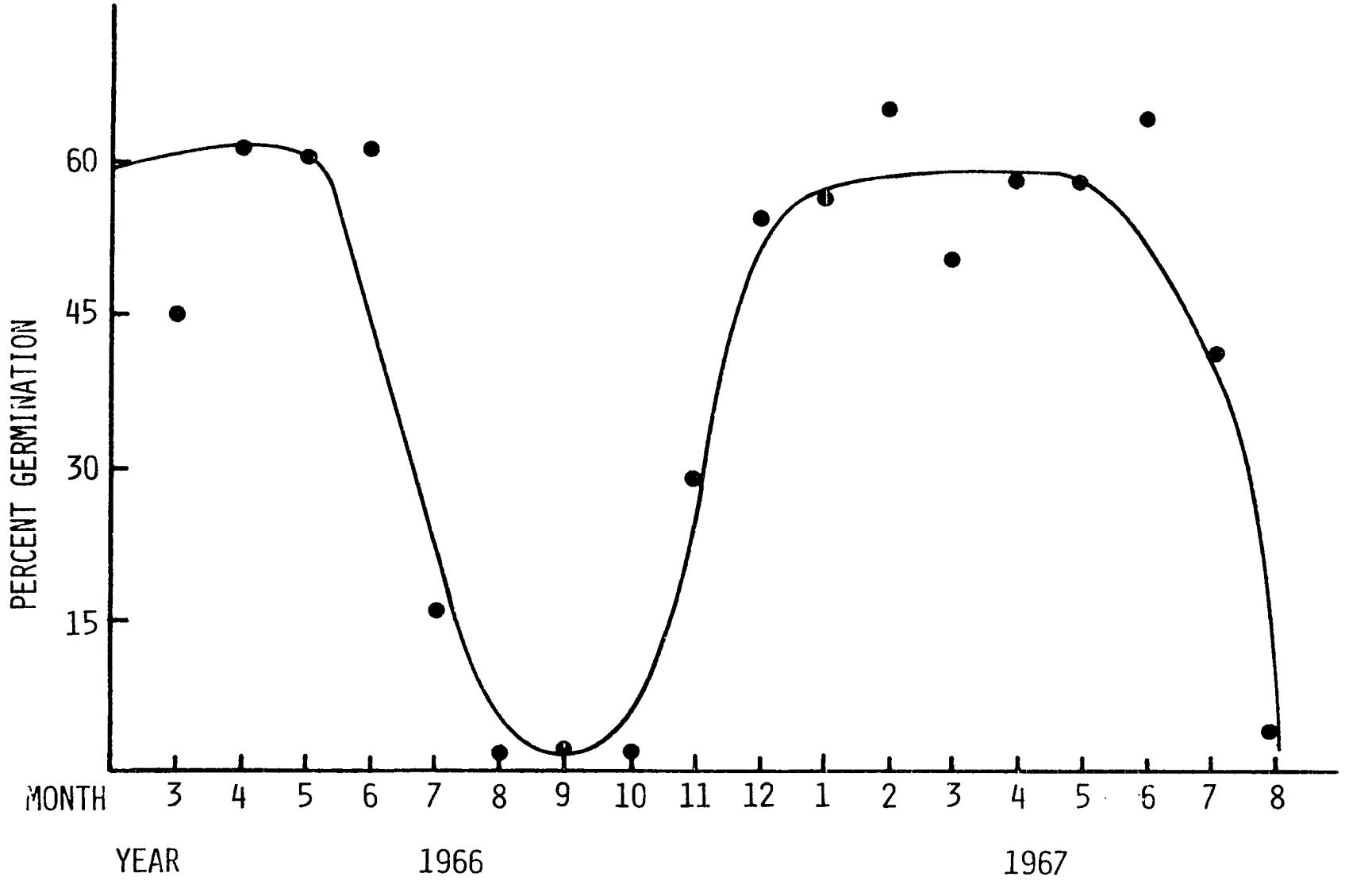
Peters and Yokum (1959) reported that acid scarification and treatment with nitrate solutions increased the germination of dormant yellow foxtail seeds. They also indicated

that newly shattered seeds were relatively impermeable to water, and suggested that this may be a factor in the dormancy of the seeds.

A study of annual germination patterns of yellow foxtail seeds under field conditions was initiated by Sells (1965), and continued by Dr. Staniforth's group at Iowa State University as reported by Ernst (1968). Soil samples infested with natural weed seed populations were obtained periodically, the weed seeds separated from the soil, and subjected to laboratory germination tests. Figure 1 shows the results of this experiment for the time period March 1966 to August 1967.

It is evident that there are annual cycles of dormancy and germination in these seeds. In their natural environment the seeds mature and fall to the ground in August or September. At this time the seeds are inherently dormant and fall germination is thus prevented. During the fall and early winter the seeds in the soil are subjected to moist low temperature conditions. It is evident from laboratory tests of field seeds that germination increases during this period, and a peak is reached in about 12 weeks. At this time in the field, the seeds do not germinate because of the low outdoor temperatures. In the spring of the year, when potential germination is at a peak, a portion of the weed seed population germinates. Those seeds which do not

Figure 1. Annual cycles of field yellow foxtail seed germination. Seeds from the field were obtained, separated from the soil, and subjected to standard laboratory germination tests


PERCENT GERMINATION
60
45
30
15
MONTH
3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8
YEAR
1966
1967

germinate gradually become dormant again in the summer months.

Yellow foxtail seeds exhibit all three categories of dormancy (innate, induced, and enforced) in succession over a period of time. Innate dormancy is an inherent property of the mature seed as it leaves the parent plant. In yellow foxtail innate dormancy prevents fall germination and is terminated by the moist cool conditions of the soil in fall and winter. As innate dormancy is terminated the germination of yellow foxtail seeds in the field during winter is prevented by low temperatures. The seeds are in a state of enforced dormancy, a condition in which limitation of the environment prevents germination. In the spring and early summer that portion of the seed population which does not germinate proceeds from a state of enforced dormancy to one of induced dormancy. Induced dormancy occurs when a non-dormant seed becomes dormant, evidently in response to the environment.

Innate dormancy is internally controlled, enforced dormancy is externally controlled, and induced dormancy is an internal control which arises from the interaction of the seed with the environment. Most research on seed dormancy has been concerned with innate dormancy or the termination of innate dormancy. The study of induced dormancy and its interaction with the environment appear to be fruitful areas for further investigation.

MATERIALS AND METHODS

Basically two types of techniques were required for this investigation. To determine the effect of exogenous germination promoters and inhibitors on the germination of yellow foxtail seeds, standard laboratory germination tests were conducted. Examination of an endogenous inhibitor or inhibitors in yellow foxtail required a large supply of pure seed, the development of stratification methods, and various extraction, separation, and assay techniques.

Seeds

The yellow foxtail seeds were obtained from seed plants grown in rows in the field at Ames, Iowa in 1968 and 1969. The batches were designated by the year of collection. The mature seeds were collected from copper and aluminum screens which were placed between the rows of the foxtail plants. Seeds were stored in plastic bags at low temperatures until used. The term "seed" will henceforth refer to the entire dispersal unit consisting of the glumes, lemma, palea, and caryopsis.

Seeds from 1969 were dormant at harvest and remained so during the entire investigation. Seeds from 1968 were also dormant at harvest but gradually became nondormant in dry low temperature storage by the summer of 1969. However, during the fall and winter of 1969 this seed lot again became

dormant.

Light sensitive Grand Rapids lettuce seeds which were used for bioassay were purchased from Macalaster Scientific Company, Nashua, New Hampshire.

Germination Tests

Seeds were placed on two blue standard germination papers in petri dishes with 13 ml of test solution and were exposed continuously to the test solution during the entire germination period. Usually four replicates of 100 seeds were used for each treatment. Most germination tests were conducted in a 15-25 C alternating temperature germinator (8 hours at 25 C and 16 hours at 15 C). Lights were on only during the eight hour period at 25 C.

In some instances germination tests were conducted on excised caryopses as well as the entire dispersal unit. Caryopses were excised by removing the lemma and palea (hull) using tweezers and razor blade under a dissecting microscope. One or two caryopses could be excised per minute using this technique.

Visible penetration of the hull by both the coleoptile and coleorhiza was used as the criterion of germination for the foxtail seeds. Germination data were recorded as the sum of ten as recommended by Timson (1965). This method gives a single numerical value which indicates both the speed and extent of germination. A running total of

the percent germination was recorded every 24 hour period for ten days, and the results summed. If 100 percent of the seeds germinated in the first 24 hour period, the sum of ten value would be 1000. Thus with this method of recording germination data there is a value range of 0 for completely dormant seeds to 1000 for rapidly germinating seeds.

Stratification

For stratification seeds were placed in petri dishes as in the germination tests and placed in cold storage at 5 C. Larger quantities of stratified seeds were required for seed extraction investigations. For these stratifications 30 gm of seeds were placed in one-half pint jars and sufficient water was supplied to result in imbibed seeds.

Chemicals

Gibberellic acid, benzyl-adenine, and kinetin were purchased from Sigma Chemical Company, St. Louis, Missouri. Gibberellin A_7 (46.36 percent) with gibberellin A_4 (53.64 percent) was purchased from Calbiochem, Los Angeles, California. The (dl)abscisic acid batch SD16108 code 1-6-0-0 was generously supplied as a gift by Shell Development Company, Modesto, California.

Seed Extraction

The extraction of an endogenous inhibitor or inhibitors from seeds consisted basically of three steps; acid fraction extraction, partial separation and purification of the acid fraction, and detection of the inhibitor by bioassay. The acid fraction of the seed extract was obtained by a methanol extraction procedure described by Milborrow (1967). Partial separation and purification of the acid fraction was conducted by paper chromatography or paper electrophoresis. Separation of the two plant hormones, gibberellic acid and abscisic acid was found to be difficult by paper chromatography, but the two were readily separated by high voltage paper electrophoresis. However, more scattering of the inhibitor band occurred with the electrophoresis technique. A bioassay utilizing light sensitive lettuce seeds was employed to detect germination inhibitors.

Extraction of the acid fraction of seed extracts consisted of the following steps.

1. Seeds were coarsely ground in a Wiley mill.

2. Ground seed material was placed in 80 percent methanol and agitated at 2 C for 6 days with two solvent changes. Usually 100 gm of seed, fresh weight, was agitated in 400 ml of 80 percent methanol.

3. The volume was reduced to approximately 50 ml by vacuum distillation at 40 C. To ensure removal of all the

methanol 500 ml of water was added and the volume again was reduced to 50 ml.

4. The water insoluble residue was separated by filtration, triturated in a saturated solution of sodium bicarbonate, then refiltered and the liquid portion combined with the original solution.

5. The pH was lowered to 5.0 with sulfuric acid. A precipitate formed at this point which separated by centrification and filtration and was discarded. The pH was then further lowered to 3.0.

6. The volume of the extract was increased to 200 or 300 ml. It was then extracted with four 10 percent volumes of ether.

7. The volume of the etheral fraction now containing the acid fraction was increased to 200 or 300 ml, and was extracted four times with 10 percent volumes of saturated sodium bicarbonate and water alternately.

8. The pH of the aqueous solution was adjusted to 3.0 with sulfuric acid, and again extracted with four 10 percent volumes of ether.

9. The ether was allowed to evaporate to dryness, and the extract was redissolved in dry ether and streaked on paper strips for electrophoresis or chromatography.

A different extraction procedure was attempted in which the ground seed material was agitated in a saturated solution

of ammonium sulfate. The pH of the solution was adjusted to 8.0 with ammonium hydroxide. The acid fraction was partitioned directly into ether from the ammonium sulfate solution which was then evaporated to dryness. This procedure was considerably shorter and resulted in much less contamination with plant pigments. However, the method proved to be less effective in extracting inhibitors from the seeds, and consequently was not extensively employed.

Paper Electrophoresis

Paper or thin layer chromatography techniques are often used to separate acidic plant hormones; however, the resolution between gibberellic acid and abscisic acid is poor. High voltage electrophoresis was found to be a very effective method of separating weak acids with slightly different pK_a values.

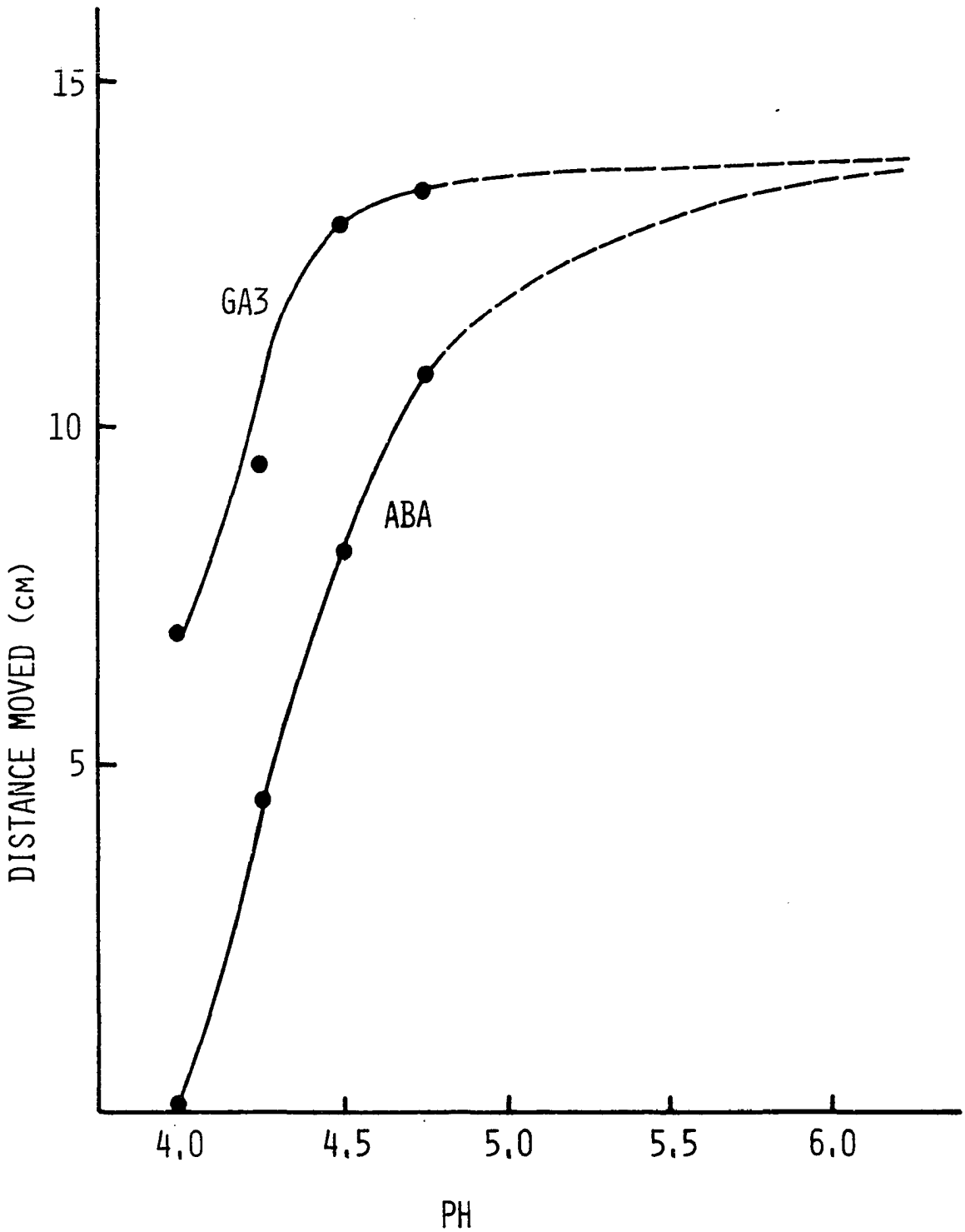
Separation of the acids was found to be very much dependent upon the pH of the buffer (electrolyte). In an electric field movement of the particles is dependent upon their electrostatic charge. The electrostatic charge of weak acids such as abscisic acid and gibberellic acid results from ionization which is controlled by the pH of the solution. An ionized weak acid has a negative electrostatic charge and thus migrates to the positive electrode in an electric field; an unionized weak acid has no charge and does not migrate. When the pH is equal to

the pK_a of the acid, it is 50 percent ionized; a higher pH results in a higher degree of ionization, a lower one in less. Thus, the pH of the buffer determines the degree of ionization of the molecule which in turn controls its migration in an electric field.

Weak acids which have slightly different pK_a values are ionized to a somewhat different degree at a particular pH, and thus migrate at different rates which results in separation. A buffer, which has a pH near or possibly between the pK_a values of the acids to be separated, results in the best resolution of the components. Figure 2 shows the separation of gibberellic acid from abscisic acid standards at various pH values. Spots of gibberellic acid and abscisic acid on the developed electrophoretogram were detected by spraying with a 0.5 percent solution of potassium permanganate. The spots appeared yellow against a purple background and persisted for only a few minutes. As the pH was increased ionization and thus migration were increased. Gibberellic acid is a slightly stronger acid than abscisic acid, and thus ionizes to a greater degree and migrates at a faster rate. At a pH of 4.5 separation was good, both compounds were moved a significant distance from the origin, and this pH was generally used.

Specifications were as follows: electrophroesis unit-Savant model FP22A flat plate with a Savant high voltage

Figure 2. The relative migration of gibberellic acid and abscisic acid during a two hour paper electrophoresis run. A citric acid--sodium hydroxide buffer at a concentration of 0.027 M was used. The voltage was 1800


15
10
5
DISTANCE MOVED (cm)
GA3
ABA
4.0
4.5
5.0
5.5
6.0
PH

power supply, paper-Whatman number 1 strips 7 x 57 cm, electric potential-1800 volts, buffer-citric acid sodium hydroxide 0.027 M with a pH of 4.5, time- 2 to 2.5 hours.

Picric acid was used as a marker for the electrophoresis runs. It migrates rapidly because it is a strong acid, and its bright yellow color permits observation of its movement during the run. Movement of gibberellic acid and abscisic acid can be indicated relative to the movement of picric acid and expressed as an R_f (picric acid) value. This is analogous to the R_f value for paper and thin layer chromatography which is based on the movement of the solvent front.

Paper Chromatography

In some instances paper chromatography was used as an alternative to paper electrophoresis for the partial separation and purification of the acid fraction of the seed extract. Although a good separation between abscisic acid and gibberellic acid is not possible with this technique, relatively little scattering of the inhibitor band occurs with paper chromatography. Thus more inhibitor was present for bioassay following separation. Since gibberellic acid does not interfere with abscisic acid detection by bioassay, separation was not essential in many experiments.

The paper strips containing the seed extract were developed by ascending chromatography until the solvent front had reached 30 cm. Whatman number 1 paper was used.

The usual solvent was n-butanol:1.5 N ammonium hydroxide (3:1) (organic phase). However, in the comparisons of the seed extract with abscisic acid standards six different solvent systems were used.

Lettuce Seed Germination Bioassay

Following the partial separation of the acid fraction of the seed extract, the developed chromatograms were examined for germination inhibitors by bioassay. Following drying, the developed chromatogram was cut into ten transverse 3.0 cm wide strips. Chromatograms were developed until the solvent front had reached a height of 30 cm, thus each cut strip represented an R_f value range of 0.1 (0.0-0.1, 0.1-0.2, etc.). When paper electrophoresis was employed to separate the acid fraction, the bioassay procedure was similar. Electrophoretograms were developed until the picric acid marker had migrated 25 cm. The electrophoretograms were cut into ten 2.5 cm wide strips.

The ten strips were placed in petri dishes and moistened with 1.5 ml of a 0.01 M citric acid-sodium hydroxide buffer with a pH of 4.5. Fifty light-sensitive Grand Rapids lettuce seeds were distributed on the moistened strips. Germination of the seeds was counted following a two day germination period at 25 C in the light.

This bioassay can be adapted to detect promoters as well as inhibitors at the same time on the chromatography

strip. Germination is counted at two different times: the first count is after a dark germination period and detects germination promoters, the second count follows a subsequent light germination period and detects germination inhibitors. Light-sensitive lettuce seeds germinate in the dark only in the presence of gibberellins; thus the dark germination period reveals promoters. The seeds normally germinate in the light except when inhibitors are present; thus the subsequent light germination period reveals inhibitors. The dark germination period was three days long and the subsequent light germination period was one additional day. When this procedure is employed the sensitivity of the bioassay to germination inhibitors is decreased and thus it was not extensively employed.

Barley Endosperm Bioassay

A barley endosperm bioassay was also employed as an alternative to the lettuce seed germination bioassay. This bioassay is based on the responses of barley aleurones to gibberellin and abscisic acid, as described by Chrispells and Varner (1967a) and Chrispells and Varner (1967b). In response to gibberellin the barley aleurone layer synthesizes and releases α-amylase. This gibberellin-induced response is inhibited by abscisic acid. Thus, the presence of abscisic acid can be detected by this system by assaying for α-amylase activity following incubation of the barley

endosperms.

Embryoless barley endosperms (variety-Himalaya) were surface sterilized in 20 percent Clorox for 20 minutes, then leached in water for three days. The leaching was conducted under sterile conditions at 2 C; the water was changed twice daily. The water leaching was conducted to remove endogenous gibberellin and reducing sugar from the barley endosperms.

Barley endosperms are incubated in the presence of gibberellic acid and a portion of the developed chromatogram paper strip. The incubation medium consisted of 1 mM acetate buffer at a pH of 4.8 containing 10 mM calcium chloride and gibberellic acid. Four ml of the incubation medium were transferred into a test tube and then autoclaved. Fifteen barley endosperms were transferred to each test tube of incubation medium. A paper strip representing 0.1 of the developed chromatogram was added to the incubation medium. The mixture was incubated for 24 hours at 25 C on a shaker.

Following incubation the bathing medium was decanted, the insoluble material washed with 3 ml of water which was added to the incubation medium. This enzyme solution was then assayed for α-amylase activity.

The enzyme solution was diluted 10 times; 0.5 ml of the diluted enzyme plus 0.5 ml of starch solution (0.45 percent Baker's soluble-potato powder for iodometry) were combined to initiate the assay reaction. The mixture was

incubated for 10 minutes at 25 C. The reaction was terminated by the addition of 1 ml of color reagent (1 percent 3,5-dinitrosalicylic acid and 30 percent potassium sodium tartrate in 0.4 N NaOH solution). Reducing sugar was determined by the method of Bernfeld (1951) as follows. The color was developed by heating 10 minutes in boiling water, the solution cooled, 8 ml of water added, and the absorbancy read in a Spectronic 20 spectrophotometer at a wave length of 540 nm.

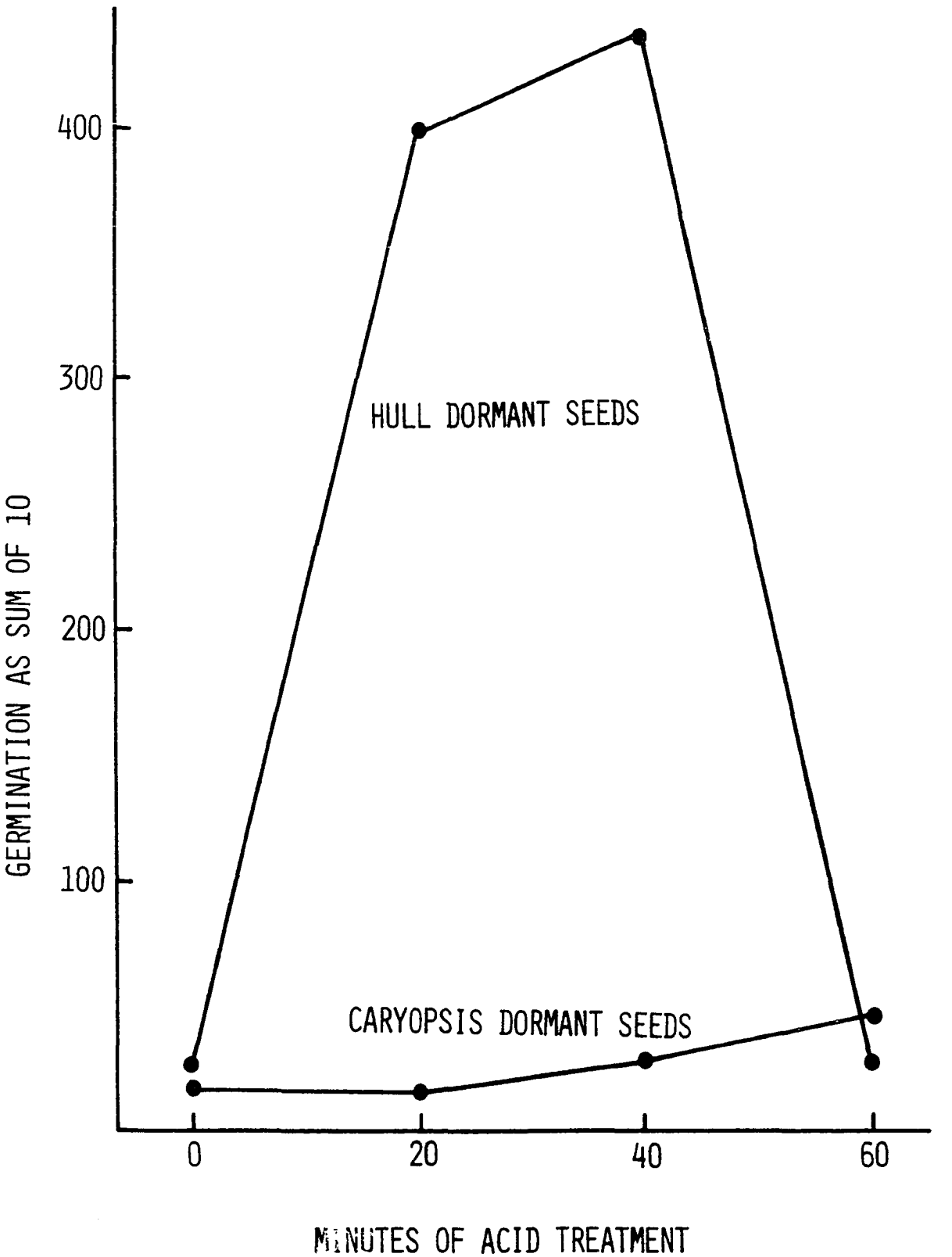
PRELIMINARY RESULTS

Nieto-Hatem (1963) indicated that there are two kinds of dormancy in yellow foxtail seeds--caryopsis and hull dormancy. In this investigation preliminary results and germination of excised caryopses substantiated that finding. Germination of excised caryopses indicated that the 1969 seed lot exhibited caryopsis dormancy; the 1968 seed lot, hull dormancy. Although the intact seeds of both lots were dormant, the excised caryopses of the 1968 lot were nondormant; the excised caryopses of the 1969 lot were dormant. Acid treatment and water leaching terminated hull dormancy, but had no effect on caryopsis dormancy. Results from water uptake studies demonstrated that dormant seeds of both types imbibed water as rapidly as nondormant seeds.

Acid Treatment

The effect of an acid treatment on the seeds exhibiting hull dormancy was grossly different from the effect on seeds exhibiting caryopsis dormancy. Dormant seeds of each seed lot were exposed to concentrated sulfuric acid for various periods of time. Figure 3 shows that the germination of the 1968 lot (hull dormancy) was greatly increased by a prior acid treatment, but the treatment had very little effect on the 1969 lot (caryopses dormancy). For the 1968 lot the effective time range for the treatment was from 20

Figure 3. The germination of hull dormant seeds and caryopsis dormant seeds after treatment with concentrated sulfuric acid for various periods of time. Germination was conducted in a 15-25 C alternating temperature germinator, and each point represents four replicates of 100 seeds


400
300
200
100
GERMINATION AS SUM OF 10
HULL DORMANT SEEDS
CARYOPSIS DORMANT SEEDS
0
20
40
60
MINUTES OF ACID TREATMENT

to 40 minutes. Evidently at treatment times beyond that range the acid penetrated the caryopsis which resulted in damage to the embryo and substantially reduced germination.

Microscopic examination of the seeds receiving an acid treatment indicated considerable damage to the lemma and palea (hull) of the seed. Results from a subsequent experiment showed that the acid treatment had no effect on the rate of water uptake. Evidently the acid treatment reduces the effectiveness of an endogenous inhibitor located in the hull either by destroying the inhibitor or increasing the leaching of the inhibitor.

Water Leaching

Water leaching was another type of experiment which demonstrated the different types of dormancy between the two seed lots. Seeds were leached in water for periods of time up to 13 days at 2 C with strong agitation. Leaching of the 1968 lot (hull dormancy) for seven days increased the germination as indicated by the sum of 10 value from 28 for the control to 402 for the leached treatment. Leaching for time periods up to 13 days for the 1969 lot (caryopsis dormancy) failed to increase germination. Evidently leaching of inhibitors from the externally located lemma and palea (hull) occurred during the treatment. This resulted in the termination of dormancy for the 1968 seed lot but not the 1969 lot.

Water Uptake

Because of the large increase in germination resulting from acid treatment, and because of indications in the literature that water impermeable seed coats are involved in the dormancy of some seeds, water uptake in yellow foxtail seeds was investigated. Peters and Yokum (1959) reported that the amount of water imbibed by various batches of yellow foxtail seeds was proportional to germination. In this investigation the correlation between water uptake and germination was examined among several seed batches including some that had been treated by various means to increase germination.

Water uptake was determined by weight differences. Seeds were weighed to the nearest 0.1 mg at 0, 6, 12, 24, and 48 hours after sowing. The seeds were placed in petri dishes on two layers of blue germination paper containing 13 ml of water. Each treatment consisted of three replicates of 25 seeds each. The experiment was conducted in the laboratory at 25 C. Moisture uptake was expressed as the percent moisture based on fresh weight.

Seeds from the 1968 batch were subjected to three different treatments which increased their germination. The treatments were: (1) soaking in concentrated sulfuric acid for 20 minutes, (2) leaching in water for 7 days at 2 C with strong agitation, and (3) stratification for 50 days. Non-

treated seed from 1969, 1968, 1967, and 1966 were included in the investigation. Germination tests were conducted in a 15-25 C alternating temperature chamber and recorded as the sum of ten.

The results shown in Table 2 indicate that there was little correlation between moisture uptake and seed germination. All of the seeds including the dormant ones imbibed a substantial quantity of water. The treatments conducted on the 1968 seeds greatly increased the germination, but there was no concurrent increase in water uptake. The acid treated seeds which germinated at a very fast rate had a slightly lower water uptake rate than the nongerminating untreated 1968 seed. Further, the water uptake of the 1966 seed, which germinated, was somewhat less than the 1967 batch which did not germinate. Thus it appears that dormant seeds imbibe water as rapidly as nondormant seeds.

Table 2. Moisture uptake and germination data for several seed batches. Moisture uptake is expressed as percent moisture of the seeds for 0, 6, 12, 24, and 48 hours. Three of the seed batches were treated as indicated to increase their germination. Standard deviations are expressed directly below the moisture and germination data

Seed batch	Percent moisture of the seeds for time periods up to 48 hours					Germination as sum of ten
	0	6	12	24	48	
1968 no treatment	8.3	26.5	29.5	30.4	32.5	28
	0.3	1.1	1.0	0.8	0.6	11
1968 stratification	5.8	29.1	31.8	32.7	34.9	253
	0.1	0.1	0.1	0.1	0.1	30
1968 acid treated	6.3	22.7	25.0	26.7	33.3	542
	2.1	2.1	1.0	1.3	1.7	15
1968 water leached	6.1	19.9	24.3	26.1	30.4	402
	1.0	1.7	0.5	1.3	1.0	30
1969	6.8	22.0	26.0	26.9	31.1	8
	0.8	2.0	2.0	1.7	1.0	6
1966	8.6	21.6	24.3	25.1	29.7	267
	1.0	0.4	1.4	0.4	2.2	10
1967	7.6	26.5	29.2	32.0	35.0	5
	2.4	2.8	1.8	0.2	0.3	4

EFFECT OF EXOGENOUS PROMOTERS AND INHIBITORS ON SEED GERMINATION

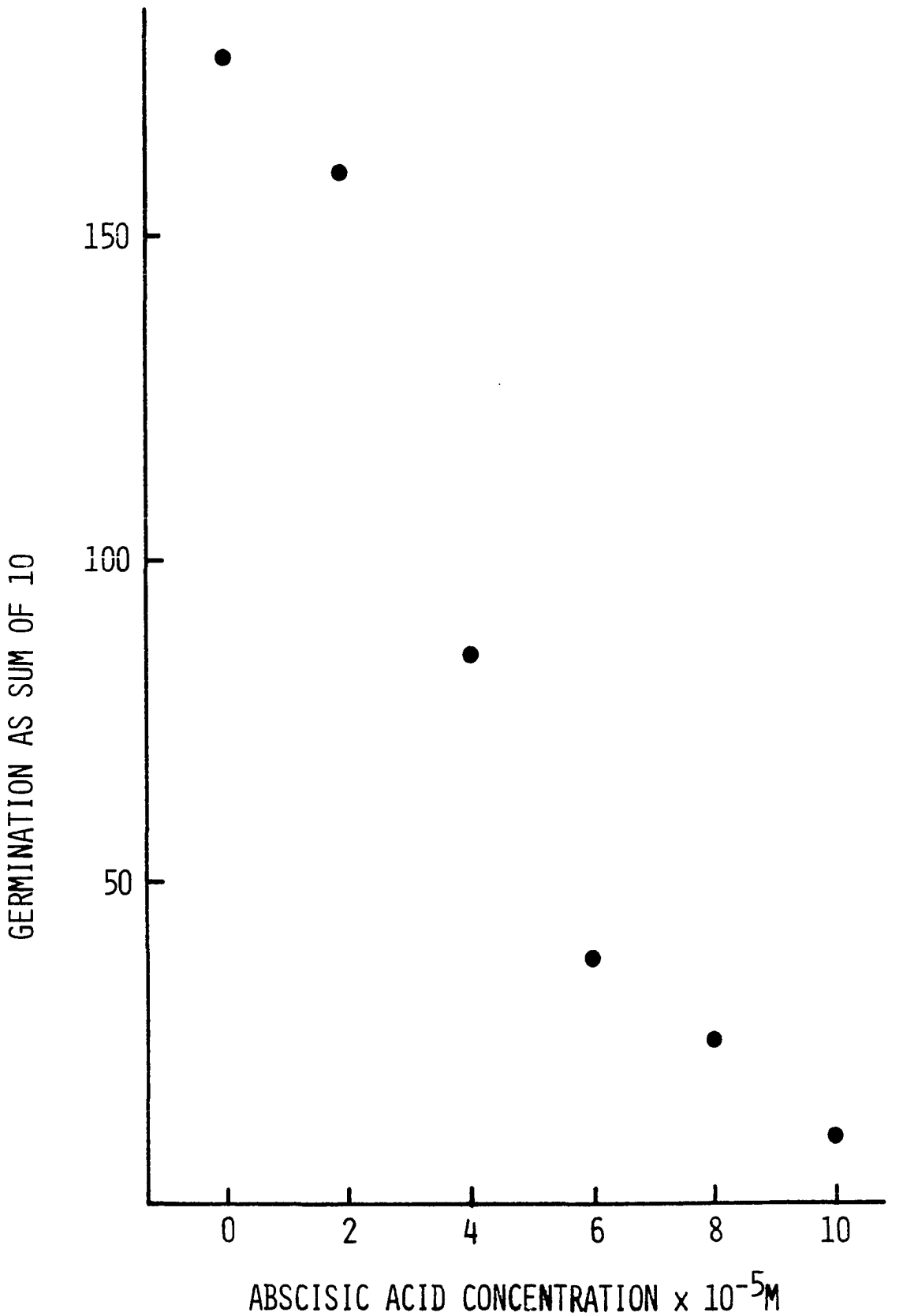
According to the promoter-inhibitor hypothesis of seed germination and dormancy, endogenous regulators control these processes. Based on that hypothesis, it would be expected that similar or identical regulators supplied exogenously would exert an influence on the control of seed germination. This section of the investigation was concerned with that expectation. The results demonstrated that several plant hormones exerted strong influences on the germination and dormancy of these seeds. Abscisic acid inhibited seed germination; the gibberellins and cytokinins promoted seed germination. The cytokinins were effective in reversing abscisic acid inhibition of germination, the gibberellins were not.

Abscisic Acid Inhibition

Synthetic (dl) abscisic acid was an effective inhibitor of seed germination in yellow foxtail when supplied in sufficient amounts. Figure 4 shows that the effective range for abscisic acid inhibition was from (1 to 10) x 10^{-5} M. A linear relationship between germination and concentration was found within that concentration range.

Exogenous abscisic acid inhibited germination in both nondormant seeds and in previously dormant seeds which had

Figure 4. The effect of abscisic acid on the germination of nondormant seeds. Germination was conducted at 25 C, and each point represents four replicates of 100 seeds

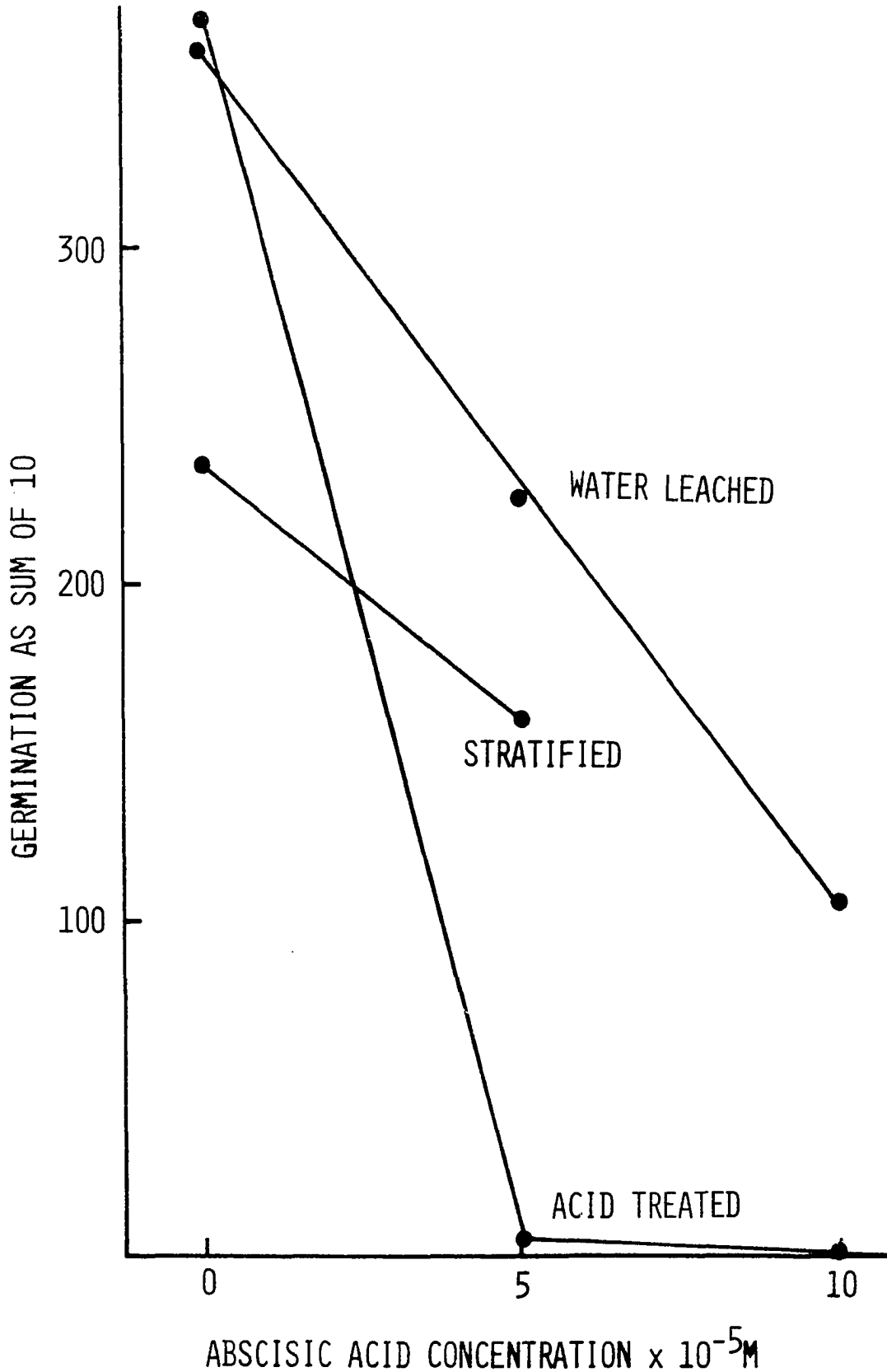

150
100
50
GERMINATION AS SUM OF 10
0
2
4
6
8
10
ABSCISIC ACID CONCENTRATION x 10^{-5}M

been exposed to a treatment which terminated dormancy. Figure 5 shows the effect of abscisic acid on seeds in which the dormancy was terminated by water leaching, stratification, or acid treatment. With each method, germination of the resulting germinable seeds was inhibited by abscisic acid. The acid treated seeds were most sensitive to abscisic acid, possibly because acid damage to the lemma and palea facilitated penetration of the inhibitor. The effects of abscisic acid were transient and free of phytotoxicity since transfer of inhibited seeds from the abscisic acid solution to water resulted in normal germination.

The effect of abscisic acid on excised caryopses was somewhat surprising. At a concentration of 1×10^{-4} M abscisic acid, the germination of intact seeds was completely inhibited. However, excised caryopses exposed to that concentration began to germinate before inhibition was manifested. The caryopses germinated to the extent that both the coleoptile and the coleorhiza had penetrated the caryopsis coat and elongated to approximately 1 mm each. Further elongation by the coleoptile and primary leaf or the radicle did not occur in the presence of abscisic acid. Thus it appears that the inhibition resulting from abscisic acid did not occur at the onset of germination, but after the germination process had begun in excised caryopses.

The effect of abscisic acid on excised embryo was very

Figure 5. The effect of abscisic acid on the germination of seeds in which dormancy had been broken by one of three laboratory techniques. Water leached seeds were agitated for seven days at 2 C with daily changes of water. Acid treated seeds were exposed to concentrated sulfuric acid for 30 minutes prior to the germination test. Stratified seeds were exposed to moist low temperature conditions for 50 days. Germination was conducted in a 15-25 C alternating temperature germinator, and each point represents two replicates of 100 seeds


300
200
100
GERMINATION AS SUM OF 10
WATER LEACHED
STRATIFIED
ACID TREATED
0
5
10
ABSCISIC ACID CONCENTRATION x 10^{-5}M

similar to that on excised caryopses. The embryos in the presence of abscisic acid began to elongate, but then were inhibited from further growth. Exogenous abscisic acid at 1×10^{-5} M inhibited the elongation of excised embryos which indicated that they are more sensitive to the inhibitor than intact seeds or excised caryopses.

The structure of vitamin A is quite similar to abscisic acid, and for this reason its effect on seed germination was examined. The acid form of the vitamin (retinoic acid) was used because of its water solubility. At 1×10^{-4} M retinoic acid had no effect on seed germination.

Gibberellin Promotion

In contrast to the effects of abscisic acid, it was difficult to demonstrate a response to gibberellin with intact seeds. Initial attempts to break dormancy or increase germination with gibberellic acid on yellow foxtail seeds were unsuccessful. Very likely a large part of the difficulty arose from the fact that the lemma and palea were impermeable to the hormone.

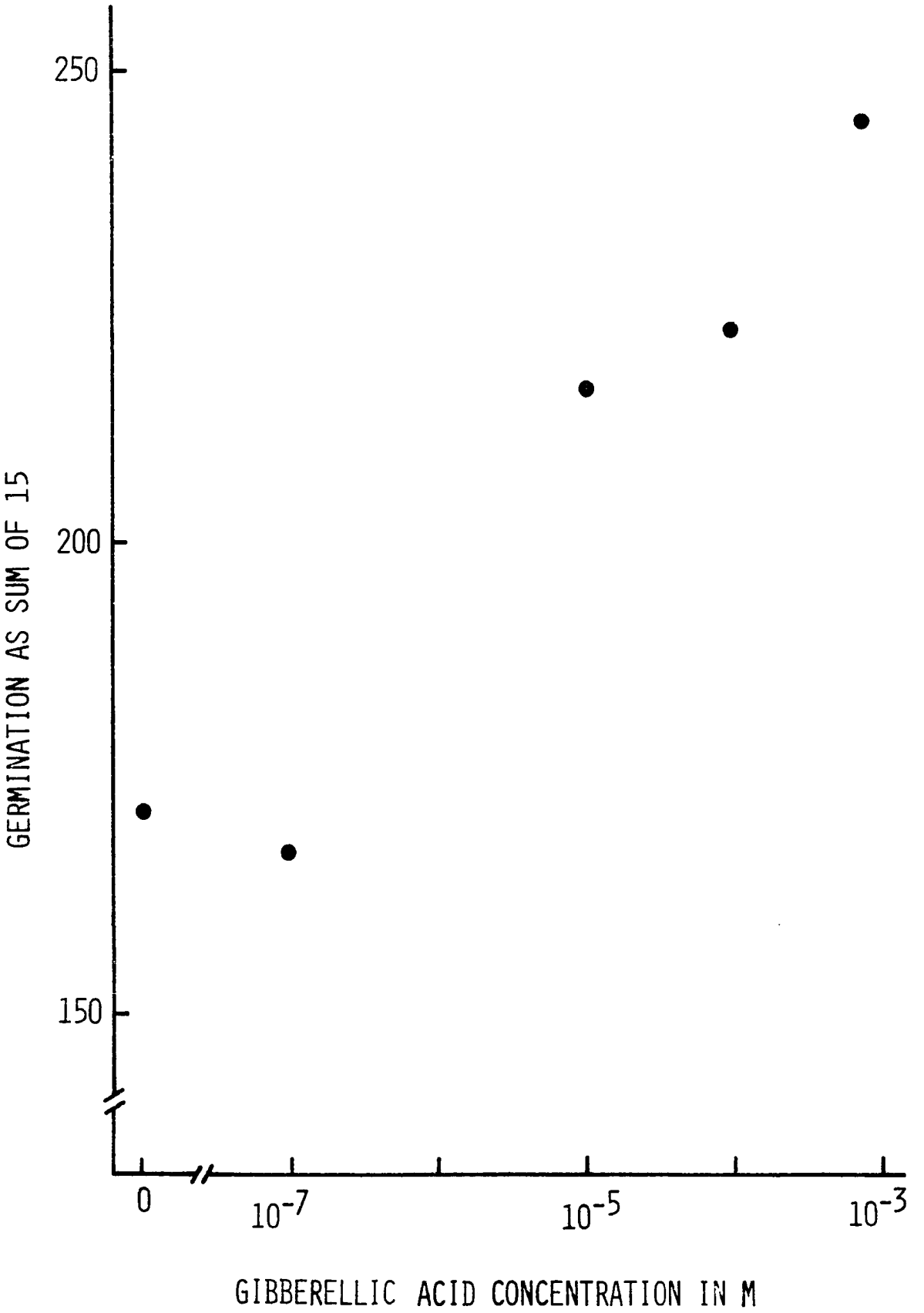
One method of testing compounds for germination promotion involves exposure of the seeds to conditions which are suboptimal for germination. Under such conditions promotion is often more readily detected. For example, gibberellin has a very strong effect on light-sensitive lettuce seed germination in the dark, but no response is

detectable in the light.

At the suboptimal temperature of 15 C yellow foxtail seeds germinated very slowly; however, in the presence of gibberellic acid an increase in the germination rate was obtained. Figure 6 summarizes the germination in the presence of various concentrations of gibberellic acid at 15 C. The magnitude of the difference between treatments was not great, but the sum of 15 values for all concentrations of gibberellic acid except 1×10^{-7} M are significantly different from the control at the one percent level of probability. With the slow germination rates at this temperature it was expedient to extend the germination period five days and thus express the data as the sum of 15. The summation method of recording germination data reflects both the rate and the extent of germination. In this case the higher value for the gibberellin treatments resulted primarily from a faster rate of germination. At the end of 20 days the germination for controls and treatments alike were between 60 and 75 percent.

Similar results were obtained when germination of non-dormant seeds was partially inhibited by an osmotic solution. Mannitol solutions of 0.2 and 0.3 M concentrations inhibited germination effectively; gibberellic acid partially overcame this inhibition. Thus germination which was inhibited by either low temperature or an osmotic solution was enhanced

Figure 6. The effect of gibberellic acid on the germination of nondormant seeds at 15 C. Each point represents four replicates of 100 seeds


250
200
150
GERMINATION AS SUM OF 15
0
10^{-7}
10^{-5}
10^{-3}
GIBBERELLIC ACID CONCENTRATION IN M

by gibberellic acid.

Gibberellic acid was ineffective in breaking the dormancy of intact yellow foxtail seeds. However, if prior to the germination test the seeds were subjected to an acid treatment, a very definite gibberellin mediated response was noted. Evidently the damage to the lemma and palea caused by the acid facilitated the entry of the hormone into the seed. Figure 7 shows the effect of gibberellic acid on dormant seeds which had previously been treated with concentrated sulfuric acid for 30 minutes. The germination as measured by the sum of 15 was linear with the logarithm of the concentration of gibberellic acid. However, even in the presence of gibberellin the germination rate was slow, and the highest germination percentage obtained was 48. In addition to the data reported in the graph, seeds were also treated in a separate experiment with gibberellin A_4 with gibberellin A_7 at a concentration of 1×10^{-4} M. This combination product is available commercially, and consists of approximately 50 percent gibberellin A_4 and 50 percent gibberellin A_7. At 1×10^{-4} M gibberellin A_4 with gibberellin A_7 the sum of 15 value was 275; this compares with a 212 value for gibberellic acid at the same concentration.

The most convincing results with gibberellin were obtained with dormant excised caryopses. Figure 8 show these

Figure 7. The effect of gibberellic acid on the germination of dormant seeds. The seeds were exposed to concentrated sulfuric acid for 30 minutes prior to the germination test. Germination was conducted in a 15-25 C alternating temperature germinator, and each point represents four replicates of 100 seeds

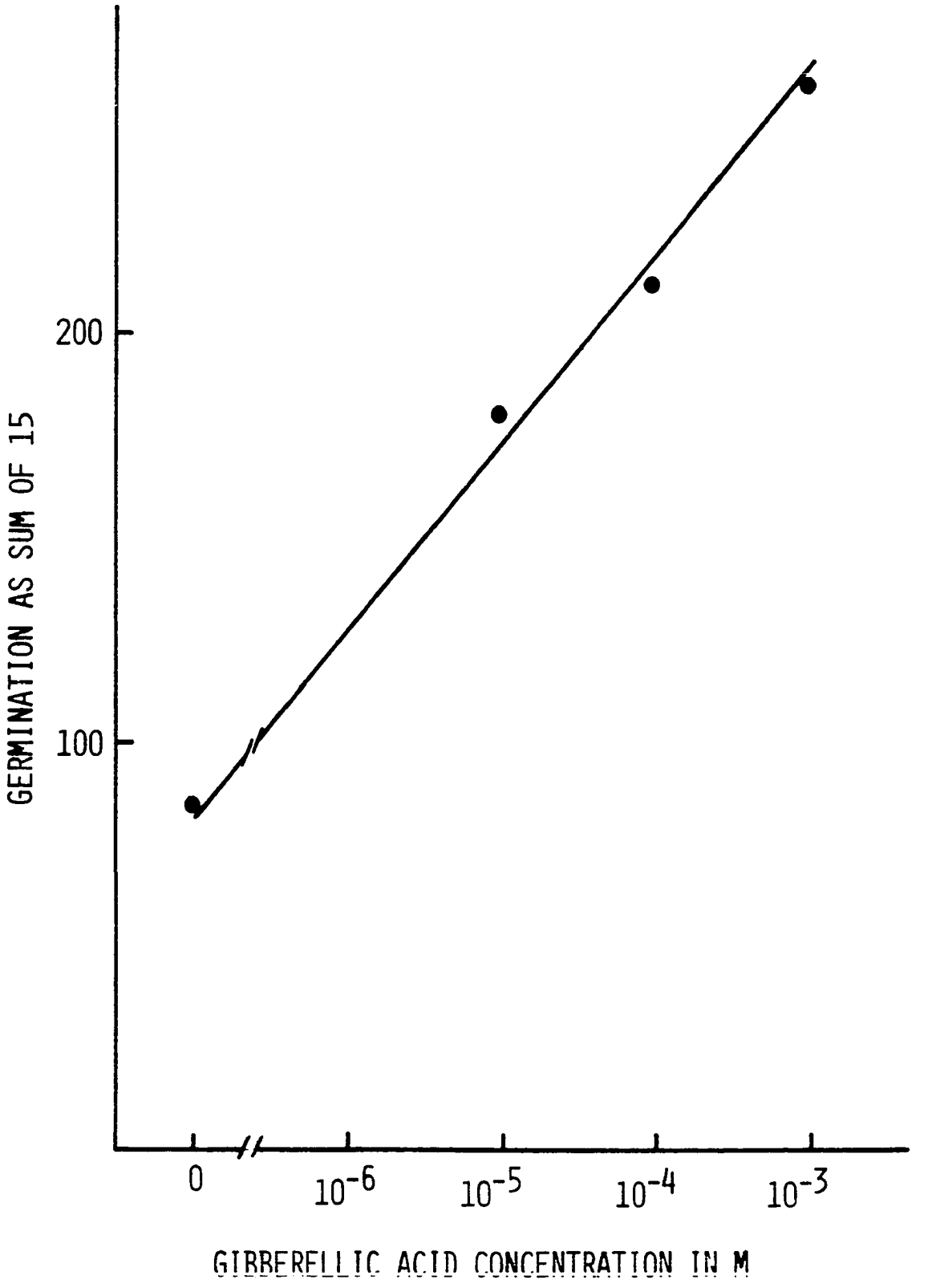
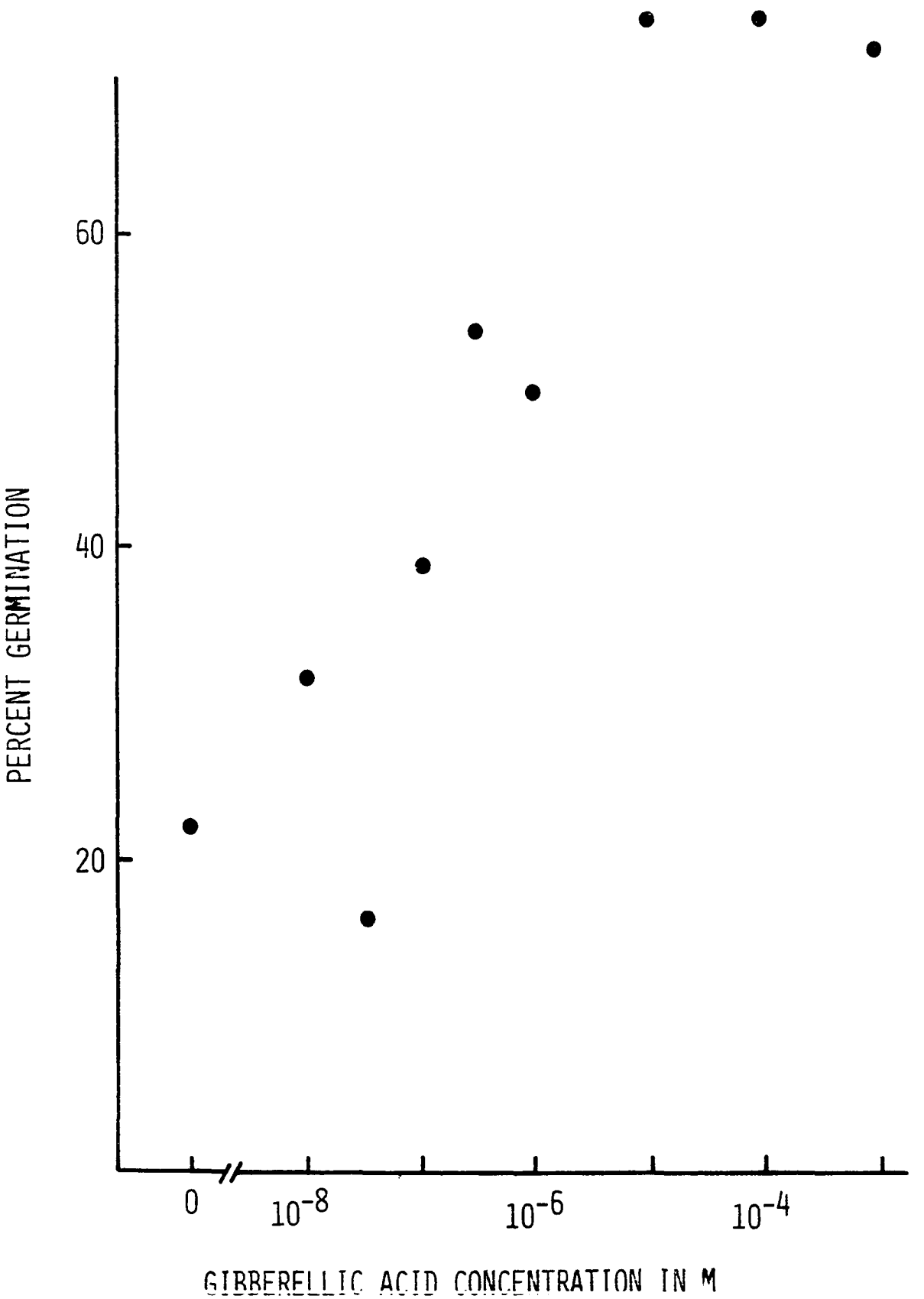

GERMINATION AS SUM OF 15
200
100
0
10^{-6}
10^{-5}
10^{-4}
10^{-3}
GIBBERELLIC ACID CONCENTRATION IN M

Figure 8. The effect of gibberellic acid on the germination of dormant excised caryopses. Germination was conducted at 25 C, and each point represents two replicates of 25 caryopses

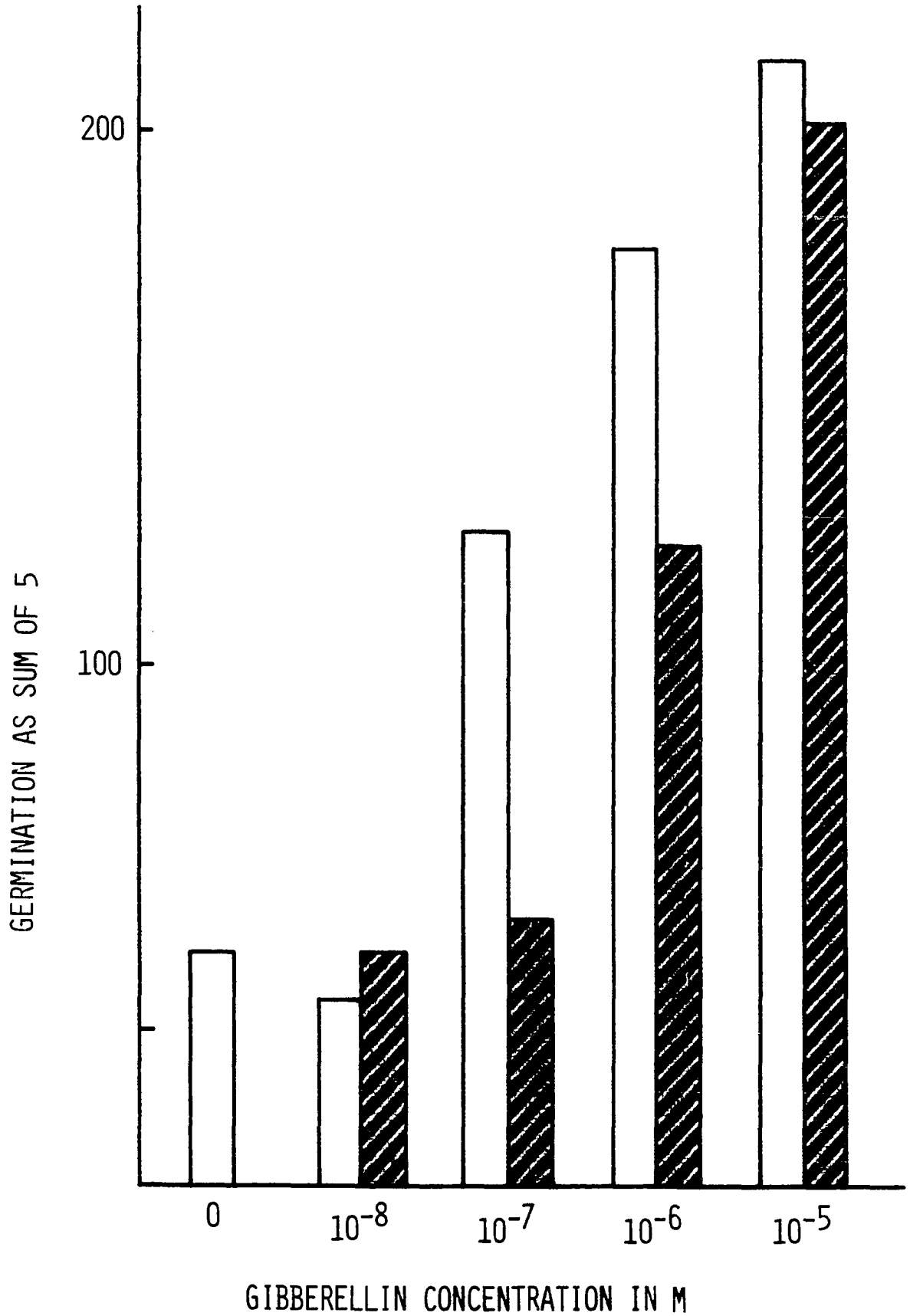

60
40
20
PERCENT GERMINATION
0
10^{-8}
10^{-6}
10^{-4}
GIBBERELLIC ACID CONCENTRATION IN M

results. With excised caryopses the gibberellin response was striking. The germination percentage was increased from 22 for the water control to 74 for the 1×10^{-5} M concentration. The effective range for gibberellin was between 1×10^{-7} M and 1×10^{-5} M. Above that range no further promotion of germination was noted. Within the effective range the response to gibberellic acid was again linear with the logarithm of the concentration.

The mixture of gibberellin A_4 with gibberellin A_7 was considerably more effective in stimulating caryopses germination than gibberellic acid, a result observed previously for whole seeds. Figure 9 shows the difference between the two treatments at several concentrations. Because of the relatively rapid germination of excised caryopses, the germination values were expressed as the sum of 5. At 1×10^{-8} M there was no effect by either treatment; however, at 1×10^{-7} M and 1×10^{-6} M gibberellin A_4 with gibberellin A_7 was considerably more effective. At the highest concentration (1×10^{-5} M) the responses were approximately equal, probably because at that concentration the responses were saturated.

The effect of two proposed gibberellin synthesis inhibitors AMO 1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperdinecarboxylate methyl chloride) and CCC (2-chloroethyl trimethylammonium chloride) on the

Figure 9. The comparison of gibberellic acid (shaded bars) and gibberellin A_4 with gibberellin A_7 (open bars) on the germination of excised caryopses at several concentrations. Germination was conducted at 25 C, and each treatment consisted of two replicates of 25 caryopses


200
100
GERMINATION AS SUM OF 5
0
10^{-8}
10^{-7}
10^{-6}
10^{-5}
GIBBERELLIN CONCENTRATION IN M

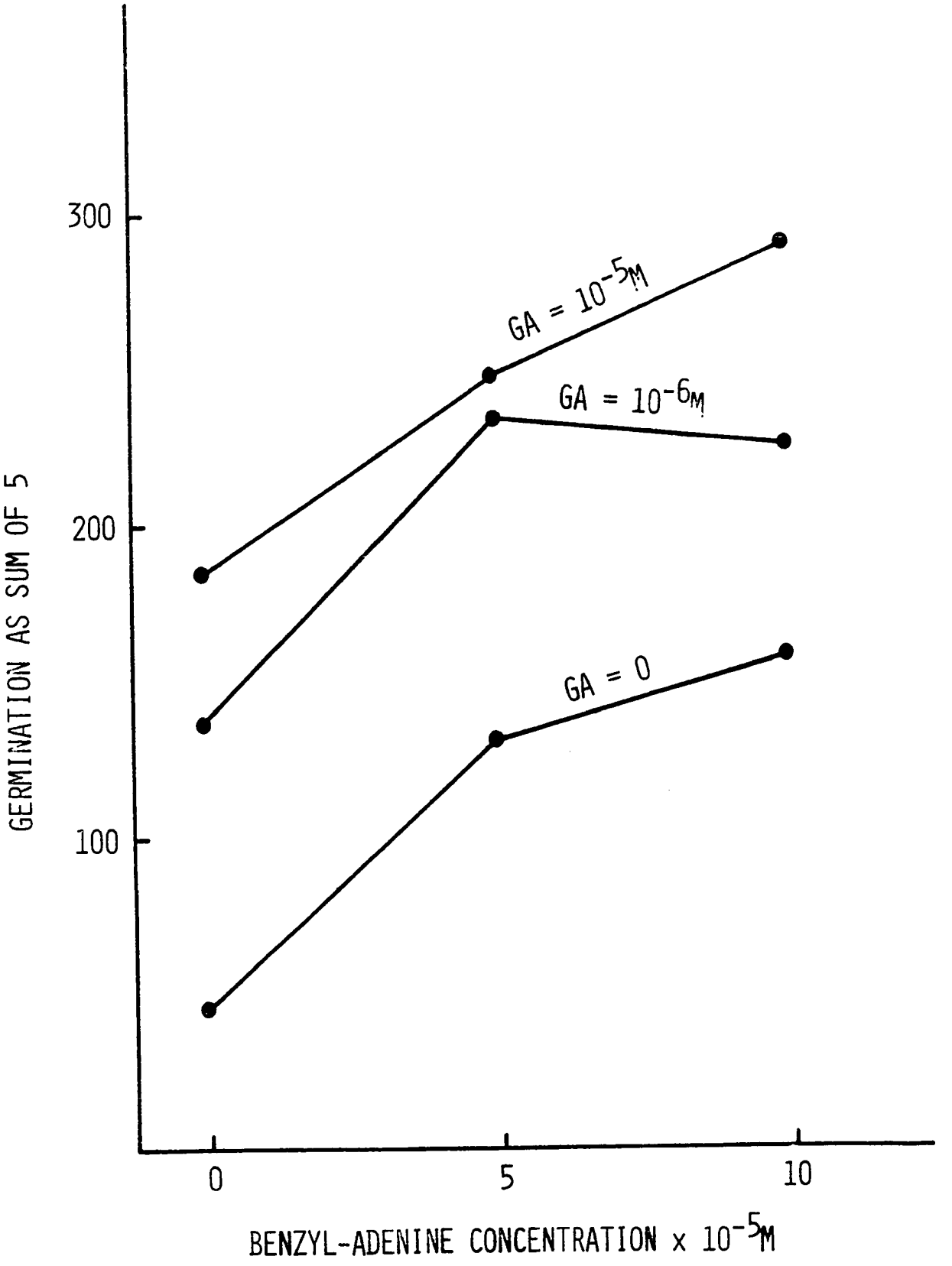
germination of yellow foxtail seeds was investigated. At concentrations up to 3×10^{-3} M for CCC and 1×10^{-3} M for AMO 1618, no effect on germination of seeds or excised caryopses was observed. These compounds also had no effect on the germination of barley seeds in which the role of gibberellin is well documented.

Gibberellin and Cytokinin Responses

Benzyl-adenine, a cytokinin, was found to be effective in promoting the germination of dormant excised caryopses. Benzyl-adenine at a concentration of 1×10^{-4} M increased the germination from 72 (sum of five value) for the control to 200. At a concentration of 1×10^{-5} no effect was observed. These data indicated that the effective range of benzyl-adenine was between (1 and 10) $\times 10^{-5}$ M, and that the germination response was proportional to the concentration rather than the logarithm of the concentration.

The effect of benzyl-adenine and gibberellin used in combinations was studied. The experiment was designed as a factorial with three concentrations of benzyl-adenine (0, 5, and 10) $\times 10^{-5}$ M and three concentrations of gibberellin A_4 with gibberellin A_7 (0, 1, and 10) $\times 10^{-6}$ M. These concentrations were within the effective range of both promoters. The results shown in Figure 10 indicated there was a strong response to both promoters. The germination response to combinations of benzyl-adenine and

Figure 10. The effect of gibberellin A_4 with gibberellin A_7 (GA) and benzyl-adenine on the germination of dormant excised caryopses. Germination was conducted at 25 C, and each treatment consisted of two replicates of 25 caryopses


300
200
100
GERMINATION AS SUM OF 5
GA = 10^{-5}M
GA = 10^{-6}M
GA = 0
0
5
10
BENZYL-ADENINE CONCENTRATION x 10^{-5}M

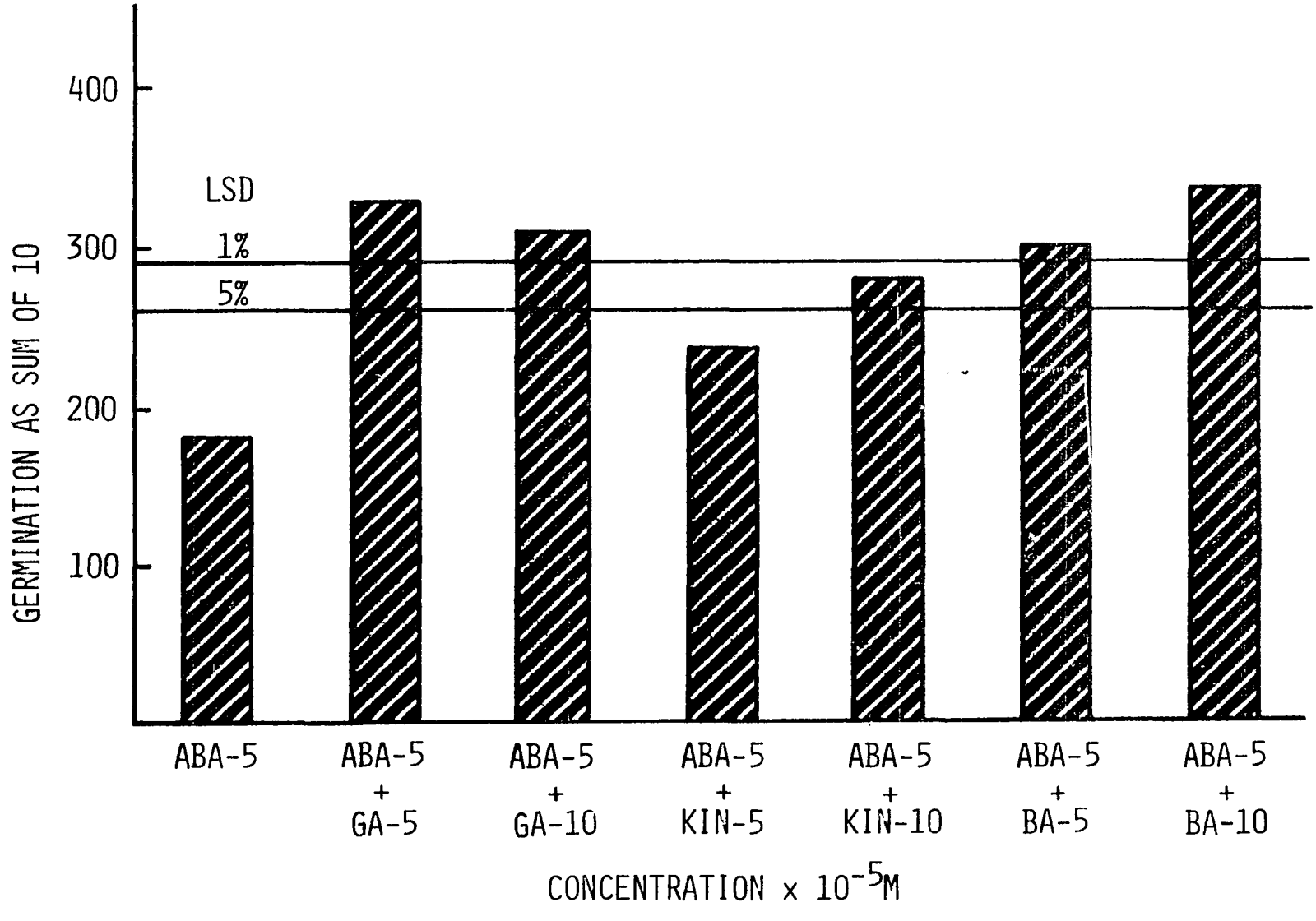
gibberellin appeared to be additive rather than synergistic.

Promoter-Inhibitor Interactions

This section of the investigation was directed toward an evaluation of the role of promoters in overcoming abscisic acid inhibition of germination. It was based on the proposal of Khan and Waters (1969) that cytokinins function in seed germination by specifically neutralizing the inhibitory effects of abscisic acid. According to their proposal gibberellins are not effective in reversing abscisic acid caused inhibition.

Gibberellic acid and two cytokinins, kinetin and benzyladenine, were examined for their effect on reversing abscisic acid inhibition. Nondormant, acid treated seeds were used which germinated rapidly and which were relatively sensitive to abscisic acid inhibition. The seeds were germinated on two layers of Whatman number 1 filter disks moistened with 3 ml of test solution. This deviation from the usual procedure was employed to conserve the abscisic acid supply. All treatments shown in Figure 11 included abscisic acid at a concentration of 5×10^{-5} M, and all except the abscisic acid control also included one of the three promoters. Each of the three promoters was tested at two concentrations, $(5 \text{ and } 10) \times 10^{-5}$ M; the lower concentration was equal to that of the abscisic acid, the higher one was double that of the inhibitor concentration. The two horizontal lines on the

Figure 11. The effect of three germination promoters gibberellic acid (GA), kinetin (KIN), and benzyl-adenine (BA) on the germination of seeds inhibited by abscisic acid (ABA). The two horizontal lines on the graph indicate least significant differences (LSD) above the abscisic acid control at the 1 and 5 percent levels. Germination was conducted at 25 C, and each treatment consisted of four replicates of 100 seeds

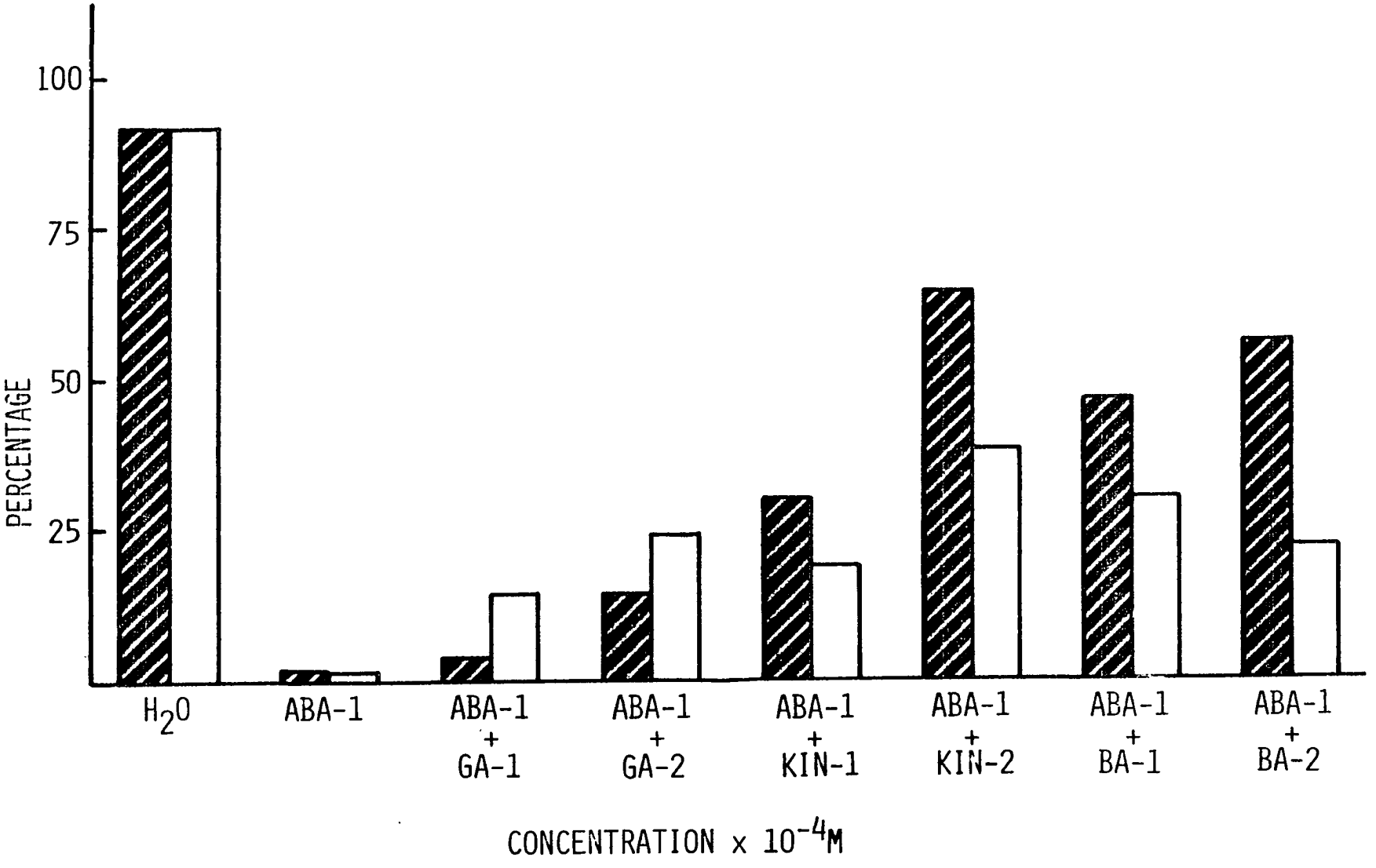

400
300
200
100
LSD
1%
5%
GERMINATION AS SUM OF 10
ABA-5
ABA-5 + GA-5
ABA-5 + GA-10
ABA-5 + KIN-5
ABA-5 + KIN-10
ABA-5 + BA-5
ABA-5 + BA-10
CONCENTRATION x 10^{-5}M

graph indicate least significant differences (LSD) above the abscisic acid control at the 1 and 5 percent levels of probability.

Abscisic acid at 5×10^{-5} decreased the germination, as indicated by the sum of 10 values, from 511 for the water control (not shown on the graph) to 184 for the inhibitor treatment. As shown in Figure 11 the three promoters reversed the abscisic acid inhibition. However, the reversal of inhibition was only partial; none of the treatments approached the 511 value for the water control. In this experiment there was very little evidence to indicate that the cytokinins were more effective than gibberellin in reversing abscisic acid inhibition of seed germination. This experiment was also run with nonacid treated seeds, and very similar results were obtained. In the second run benzyl-adenine at the high concentration was considerably more effective than the other promoters in reversing the inhibition.

A more striking demonstration of promoter-inhibitor interaction was obtained with excised caryopses. The results are summarized in Figure 12 and are presented as percentages. As indicated previously abscisic acid at 1×10^{-4} M did not inhibit completely caryopses germination, but prevented elongation of both ends of the embryonic axis beyond 1 mm. The effects of promoters on the reversal of the inhibition of elongation were examined. Again each of the three pro-

Figure 12. The effect of three germination promoters gibberellic acid (GA), kinetin (KIN), benzyl-adenine (BA) on the germination of excised caryopses inhibited by abscisic acid (ABA). The shaded bars indicate the percent of coleoptiles elongated beyond 5 mm, and the open bars show the percent of the radicles that penetrated the coleorhiza. Germination was conducted at 25 C for 10 days, and each treatment represents two replicates of 25 caryopses


100
75
50
25
PERCENTAGE
H2O
ABA-1
ABA-1 + GA-1
ABA-1 + GA-2
ABA-1 + KIN-1
ABA-1 + KIN-2
ABA-1 + BA-1
ABA-1 + BA-2
CONCENTRATION x 10-4M

moters were employed at two concentrations; the lower one equal to the abscisic acid concentration, and the higher one double the inhibitor concentration. Two phenomena were recorded and shown on the graph: elongation of the coleoptile and primary leaf beyond 5 mm, and penetration of the coleorhiza by the radicle. At each treatment the shaded bar indicates coleoptile elongation, the open bar radicle penetration. It was evident from the results that the two cytokinins were effective in reversing the inhibition of coleoptile elongation. In each case the effect was greater at the high concentration. Radicle penetration of the coleorhiza was also increased over that of the abscisic acid control; however, the radicles that did penetrate failed to undergo further elongation. The elongation of the coleoptiles that resulted from cytokinin treatment extended up to 2.5 cm, but the radicles not beyond 1 to 2 mm. Gibberellic acid was only very slightly effective in reversing abscisic acid inhibition. Thus it appears that cytokinins are effective in reversing the inhibition of coleoptile elongation caused by abscisic acid, but only a slight effect was noted at the other end of the embryonic axis.

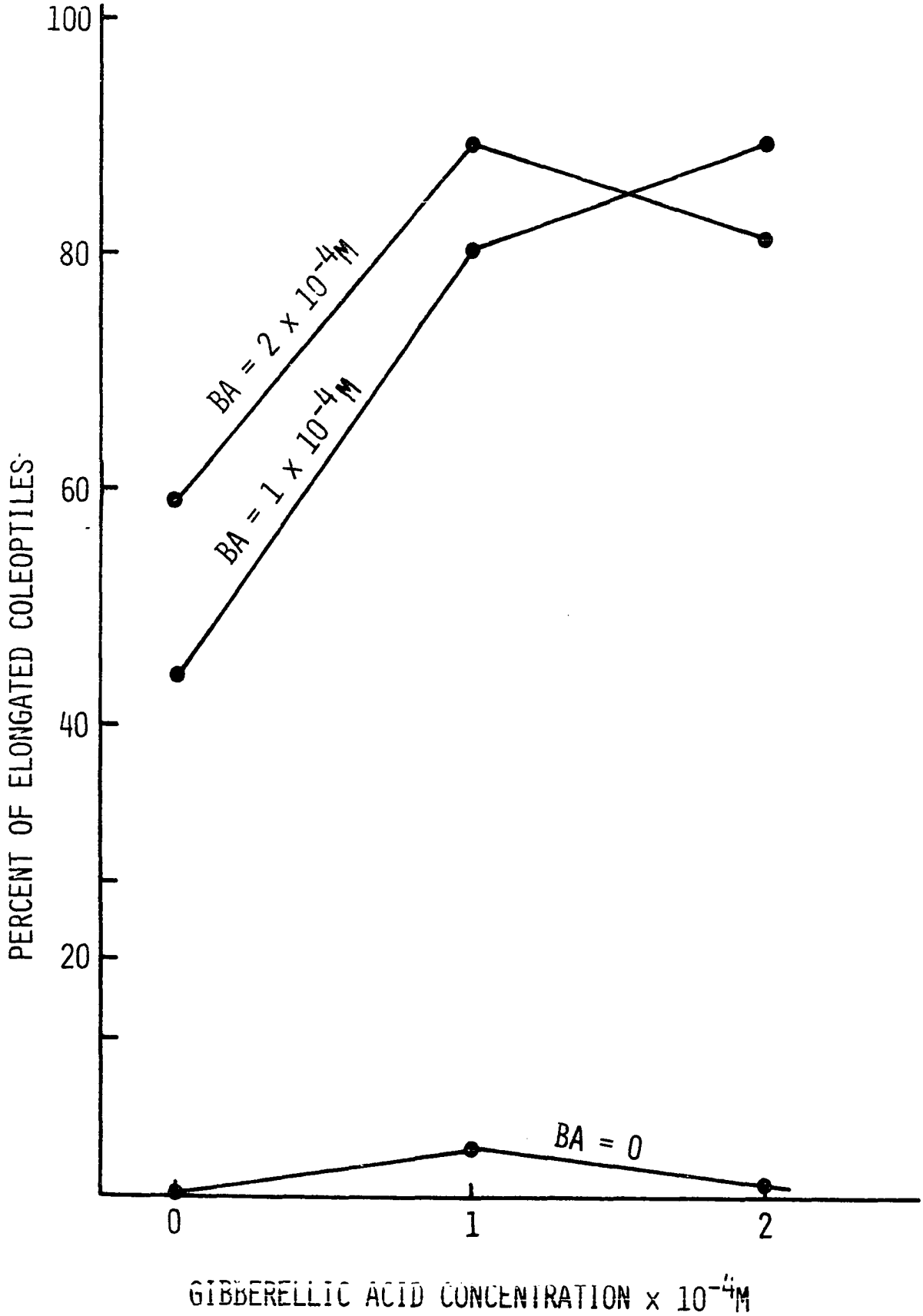
A further examination of promoter-inhibitor interactions involved the use of benzyl-adenine and gibberellic acid in combinations, rather than separately as in the

previous experiment, on abscisic acid-inhibited excised caryopses. Concentrations of the three hormones used were the same as in the previous experiment. All treatments received abscisic acid at 1 x 10^{-4} M; the benzyl-adenine and gibberellic acid were applied in a factorial manner with three concentrations of each (0, 1, and 2) x 10^{-4} M.

As noted in the previous experiment the effect of the two promoters was primarily on the coleoptile and primary leaf end of the embryonic axis. A small increase in the number of radicle that penetrated the coleorhiza was noted for the gibberellin and benzyl-adenine treatments; however, following penetration these radicles failed to elongate. The effect on coleoptile elongation is shown in Figure 13. These results clearly indicate that gibberellic acid alone had little or no effect, benzyl-adenine alone had a pronounced effect, and gibberellic acid increased the effectiveness of benzyl-adenine.

Separately or in combination gibberellic acid and benzyl-adenine appeared to be ineffective in reversing abscisic acid inhibition of the elongation at the radicle end of the embryonic axis. However, the two promoters are very effective in reversing inhibition of coleoptile and primary leaf elongation, and the effect appeared to be synergistic.

Figure 13. The effect of two germination promoters gibberellic acid (GA) and benzyl-adenine (BA) on the elongation of coleoptiles of excised caryopses inhibited by abscisic acid at a concentration of 1×10^{-4} M. Coleoptiles elongated beyond 5 mm at six days were counted. Germination was conducted at 25 C and, each treatment consisted of two replicates of 25 caryopses


100
80
60
40
20
PERCENT OF ELONGATED COLEOPTILES
BA = 2 x 10-4M
BA = 1 x 10-4M
BA = 0
0
1
2
GIBBERELLIC ACID CONCENTRATION x 10-4M

EXTRACTION AND CHARACTERIZATION OF AN ENDOGENOUS INHIBITOR

An endogenous inhibitor was extracted from yellow foxtail seeds and partially characterized by comparison with synthetic abscisic acid. A conventional technique of characterizing plant hormones consists of chromatographing the plant extract on paper or on thin layer plates and then subjecting portions of the developed chromatogram to an appropriate bioassay. Endogenous compounds present in plant extracts may be compared to hormone standards in this manner. Thus, both the chromatography behavior and its effect on the bioassay of the extracted compound may be compared with the standard. By varying the chromatographic solvent and bioassay several comparisons can be made. This technique is known as co-chromatography.

In this investigation comparisons were made between an inhibitor obtained from seed extracts and synthetic abscisic acid. In addition to paper chromatography with several solvent systems, seed extracts were separated by paper electrophoresis and compared to abscisic acid standards. Three different bioassays were employed--lettuce seed germination, the production of α-amylase by barley endosperms, and yellow foxtail seed germination.

Because the dormancy of yellow foxtail seeds can be broken by a period of stratification, the effect of stratification on endogenous inhibitor level was examined. Com-

parisons of standards with seed extracts were also made in an attempt to determine the approximate level of natural inhibitor present in the seeds.

Co-chromatography

Seed extracts were compared with abscisic acid by paper chromatography in six different solvent systems. The chromatograms were developed until the solvent front reached 30 cm from the origin, they were then dried, and cut into ten tranverse strips. The bioassay consisted of lettuce seeds germinated in a citric acid-sodium hydroxide buffer (0.01 M at a pH of 4.5) on the strips in petri dishes. After germination for two days in the light at 25 C, germination percentages were recorded. The presence of the endogenous inhibitor on a particular strip was revealed by inhibition of lettuce seed germination.

These comparisons are shown in Figures 14a and 14b. The seed extracts were equivalent to 10 grams of fresh weight of seeds; 5 µg of abscisic acid was used for the standard. The comparisons indicated that the chromatographic behavior of the extracted inhibitor was very similar to that of the abscisic acid control for all six solvent systems. With solvent system 3 (chloroform:hexane:water, 3:15:2) the abscisic acid standard and endogenous inhibitor did not move from the origin.

Figure 14a. A comparison between the chromatographic behavior of an inhibitor from seed extracts (upper row) and abscisic acid (lower row) conducted with the lettuce seed germination bioassay. Each bar in this graph represents the germination of lettuce seeds on a portion of the developed chromatogram representing 0.1 of the entire chromatogram. Similar representations are used in forthcoming histograms. The following developing solvents were used: (1) butanol:1.5 N ammonium hydroxide (3:1) (organic phase), (2) isopropanol:15 N ammonium hydroxide:water (8:1:1), (3) chloroform: hexane:water (3:15:2) (organic phase). The extracts were the equivalent of 10 gms of seed fresh weight, and 5 µg of abscisic acid was used as the standard

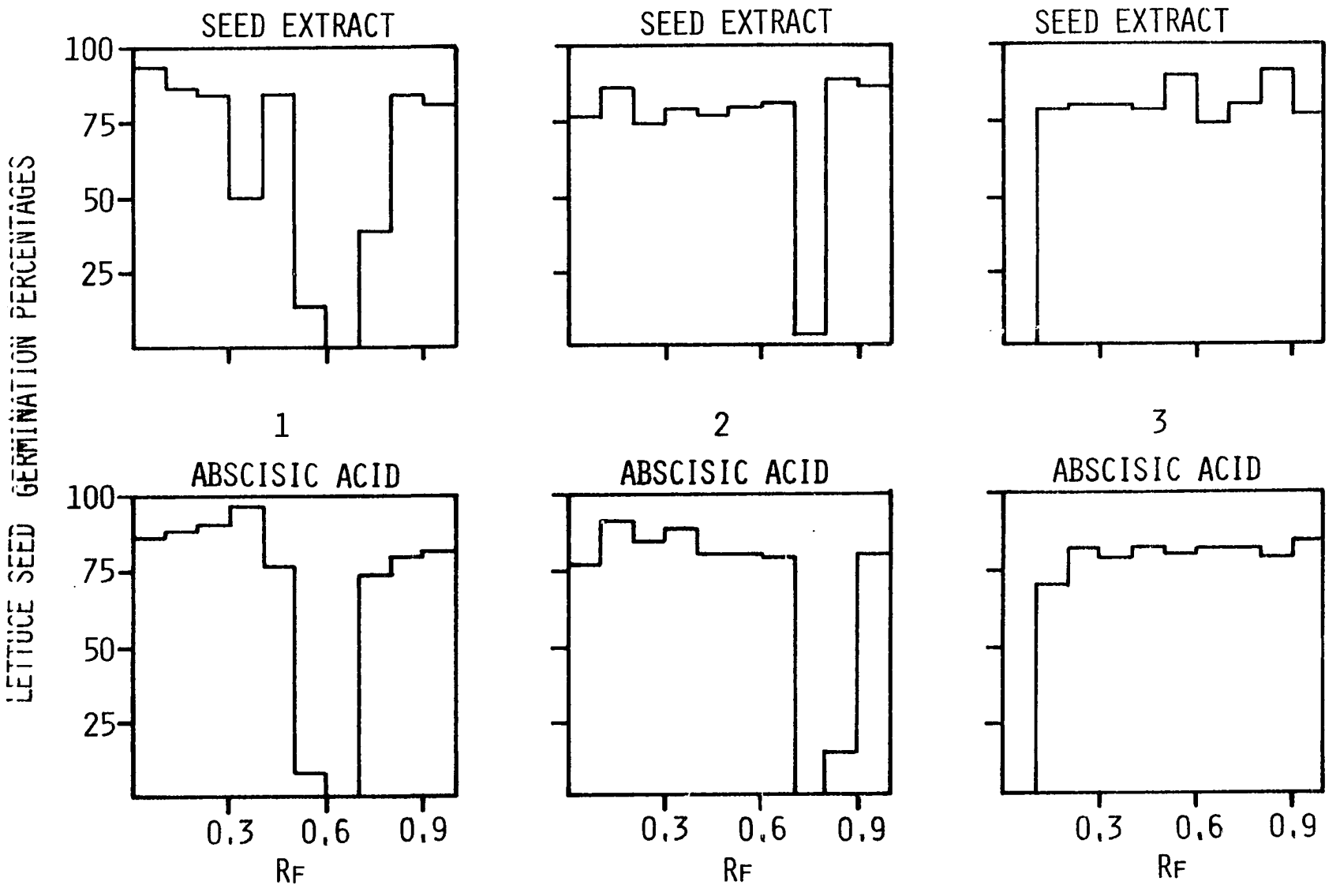
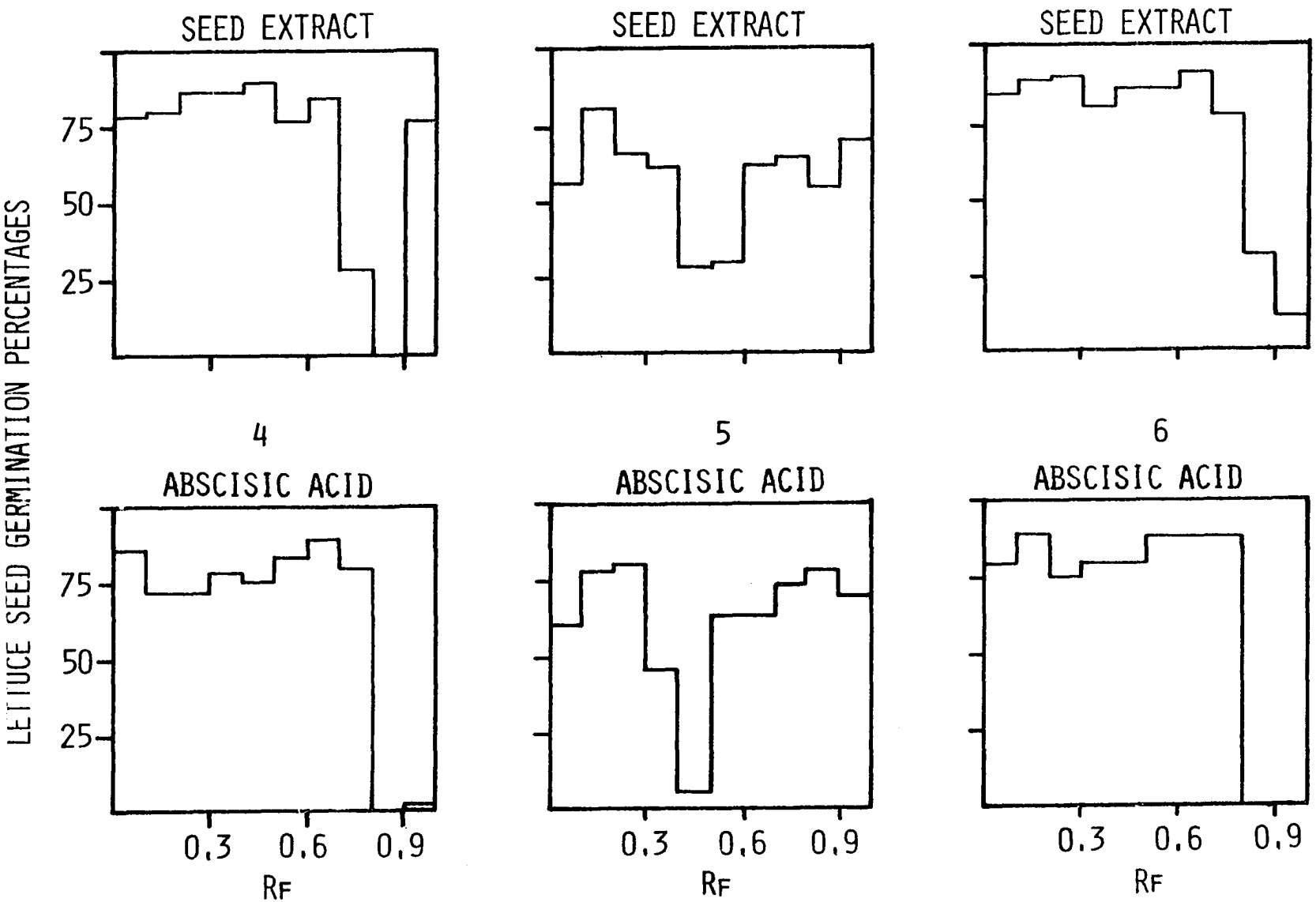

SEED EXTRACT
SEED EXTRACT
SEED EXTRACT
100
75
50
25
LETTUCE SEED GERMINATION PERCENTAGES
1
2
3
ABSCISIC ACID
ABSCISIC ACID
ABSCISIC ACID
100
75
50
25
0.3
0.6
0.9
RF
0.3
0.6
0.9
RF
0.3
0.6
0.9
RF

Figure 14b. A comparison between the chromatographic behavior of an inhibitor from seed extracts (upper row) and abscisic acid (lower row) conducted with the lettuce seed germination bioassay. The following developing solvents were used: (4) butanol:pentanol:ethanol:water (2:2:5:8), (5) pentanol:1.5 N ammonium hydroxide (3:1)(organic phase), (6) diethyl ether:methanol:acetic acid (50:50:0.1). The extracts were the equivalent of 10 gms of seed fresh weight, and 5 µg of abscisic acid was used as the standard


SEED EXTRACT
SEED EXTRACT
SEED EXTRACT
LETTUCE SEED GERMINATION PERCENTAGES
75
50
25
4
5
6
ABSCISIC ACID
ABSCISIC ACID
ABSCISIC ACID
75
50
25
0,3
0,6
0,9
RF
0,3
0,6
0,9
RF
0,3
0,6
0,9
RF

Co-electrophoresis

To further substantiate the similarity between the extracted inhibitor and abscisic acid, co-electrophoresis was conducted. This method of comparison was considerably different from chromatography because migration and separation during electrophoresis was dependent on the electrostatic charge of the molecule. The pH of the electrolyte (buffer) determines the electrostatic charge of the molecule and thus the rate of migration of weak acids in an electric field. Three different pH's were employed in the comparisons. The electrophoretograms were developed until the picric acid marker had migrated a distance of 25 cm. Figure 15 shows the results obtained with co-electrophoresis. As with co-chromatography the movement of the seed extract component and abscisic acid standards was very similar. For the electrophoresis run at a pH of 6.0, 20 gms of seed extract were used instead of the usual 10 gms. At this pH the endogenous inhibitor migrated a greater distance and thus more scattering occurred which required a larger starting quantity of extract. The location of the inhibitory areas for the six paper chromatography solvent systems and three paper electrophoresis runs are summarized in Table 3.

Figure 15. A comparison between the electrophoretic behavior of an inhibitor from seed extracts (upper row) and abscisic acid (lower row) conducted with the lettuce seed germination bioassay. The electrophoretic runs were made at three different pH values. The extracts were the equivalent of 10 gms of seed fresh weight except for the run at a pH of 6.0 where 20 gms were used. Ten µg of abscisic acid was used as the standard

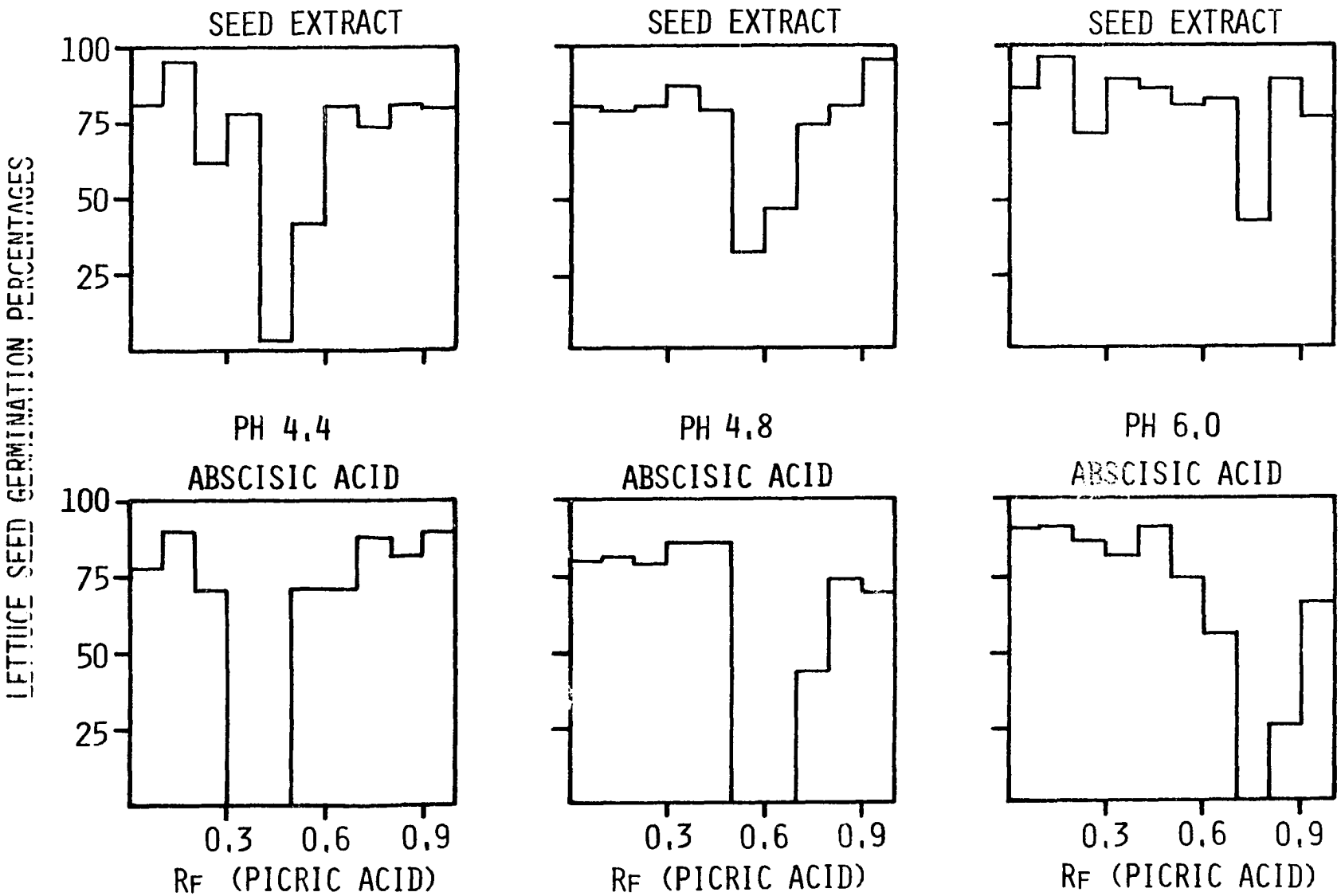

SEED EXTRACT
SEED EXTRACT
SEED EXTRACT
LETTUCE SEED GERMINATION PERCENTAGES
100
75
50
25
PH 4.4
ABSCISIC ACID
PH 4.8
ABSCISIC ACID
PH 6.0
ABSCISIC ACID
0.3
0.6
0.9
RF (PICRIC ACID)

Table 3. A summary of the comparisons between an inhibitor from seed extracts and abscisic acid standards. Results from six paper chromatography runs each with a different solvent system and three paper electrophoresis runs each at a different pH are tabulated. The data were compiled from Figures 14a, 14b, and 15

Paper chromatography		
	R_f value of inhibition	
Solvent system	Seed extract	Abscisic acid
n-butanol:1.5 N NH_4OH (3:1) (organic phase)	0.5-0.7	0.5-0.7
isopropanol:15 N NH_4OH:water (8:1:1)	0.7-0.8	0.7-0.9
Chloroform:hexane:water (15:75:10) (organic phase)	0.0-0.1	0.0-0.1
n-butanol:n-pentanol:ethanol:water (2:2:5:8)	0.7-0.9	0.8-1.0
n-pentanol:1.4 N NH_4OH (3:1)	0.4-0.6	0.4-0.5
Diethyl ether:methanol:acetic acid (50:50:0.1)	0.8-1.0	0.8-1.0

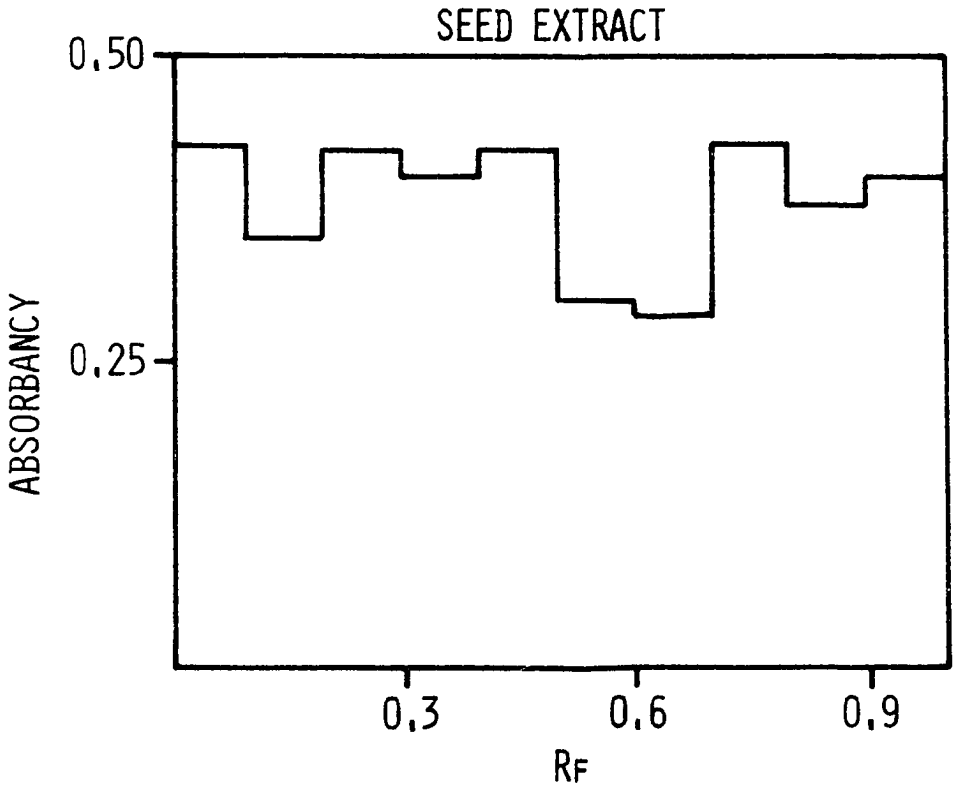
Paper electrophoresis		
	R_f (picric acid) value of inhibition	
Buffer pH	Seed extract	Abscisic acid
4.4	0.4-0.6	0.4-0.6
4.8	0.5-0.7	0.5-0.8
6.0	0.7-0.8	0.7-0.9

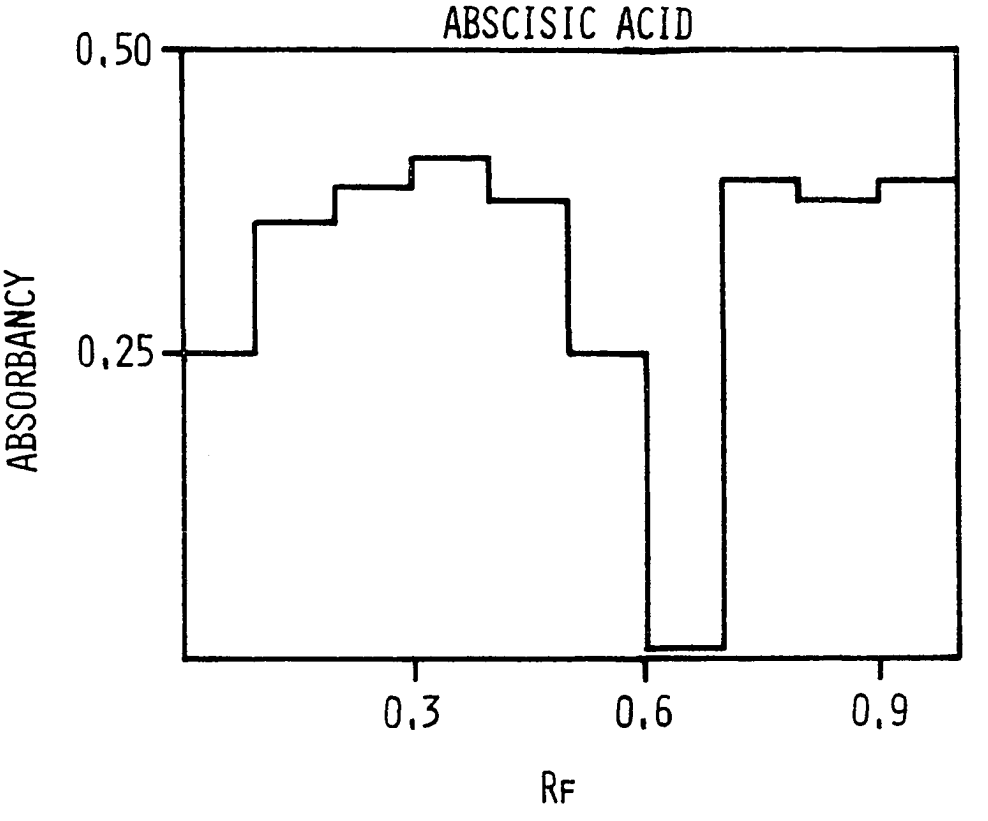
Barley Endosperm Bioassay

The natural inhibitor from the seed extract was also compared to the abscisic acid standard by the use of another bioassay. Chrispells and Varner (1967b) reported that the gibberellin-induced production of α-amylase in barley endosperms can be inhibited by abscisic acid. Thus barley endosperm may be employed as a bioassay for abscisic acid. Prior to the bioassay, the extract and the abscisic acid standard were subjected to paper electrophorcsis followed by paper chromatography. This dual separation procedure resulted in a higher degree of purity of the extract before it was employed in the bioassay. Much of the extract contamination remains at the origin during the electrophoresis, and thus is eliminated. Following the electrophoresis run a 9 cm band centered on the abscisic acid band was eluted with ether and then streaked on paper for the chromatography.

Each 0.1 portion of the developed chromatogram was placed in a test tube containing barley endosperms, gibberellic acid, and incubation media. This mixture was incubated for 24 hours and then assayed for α-amylase activity. The results are shown in Figure 16. On the histogram the areas of inhibition of α-amylase production for the seed extract were very similar to those of the abscisic acid standard. Inhibition occurred at an R_f value of 0.5-0.8 for the standard and 0.6-0.8 for the seed extract.

Figure 16. A comparison between the chromatographic behavior of an inhibitor from a seed extract and abscisic acid conducted with the barley endosperm bioassay. The following developing solvent was used: butanol:1.5 N ammonium hydroxide (3:1) (organic phase). The extract was the equivalent of 30 gms of seed fresh weight, and 10 µg of abscisic acid was used as the standard


SEED EXTRACT
0,50
0,25
ABSORBANCY
0,3
0,6
0,9
RF


ABSCISIC ACID
0,50
0,25
ABSORBANCY
0,3
0,6
0,9
RF

Yellow Foxtail Seed Germination Bioassay

The effect of the extracted inhibitor on yellow foxtail seed germination was examined by the methods employed in the lettuce seed bioassay. As in the previous experiment, the seed extract was separated by paper electrophoresis followed by paper chromatography. The extract used was equivalent to that obtained from 40 gms of fresh weight seeds. The seeds used in the germination test were treated with concentrated sulfuric acid for 30 minutes prior to the germination test to increase hull permeability to the inhibitors. Germination was conducted at 25 C, and the data recorded as the sum of 5.

The results shown in Figure 17 indicate an area of inhibition from the extract with the same R_f value as the abscisic acid inhibition band. These results show that the inhibitor extracted from yellow foxtail seeds suppressed the germination of that species.

Stratification and Inhibitor Level

Seed dormancy in yellow foxtail can be broken by stratification. As shown in Figure 18 the germination potential of yellow foxtail seeds can be greatly increased by a stratification treatment. The increase in germination was proportional to the stratification time, and at the end of 10 weeks of the treatment the seeds germinated at a very high level. Stratification of the seeds was conducted under moist condition at 5 C.

Figure 17. A comparison between the chromatographic behavior of an inhibitor from a seed extract and abscisic acid conducted with the yellow foxtail seed germination bioassay. The following developing solvent was used: butanol:1.5 N ammonium hydroxide (3:1) (organic phase). The extract was the equivalent of 40 gms of seed fresh weight, and 10 µg of abscisic acid was used as the standard

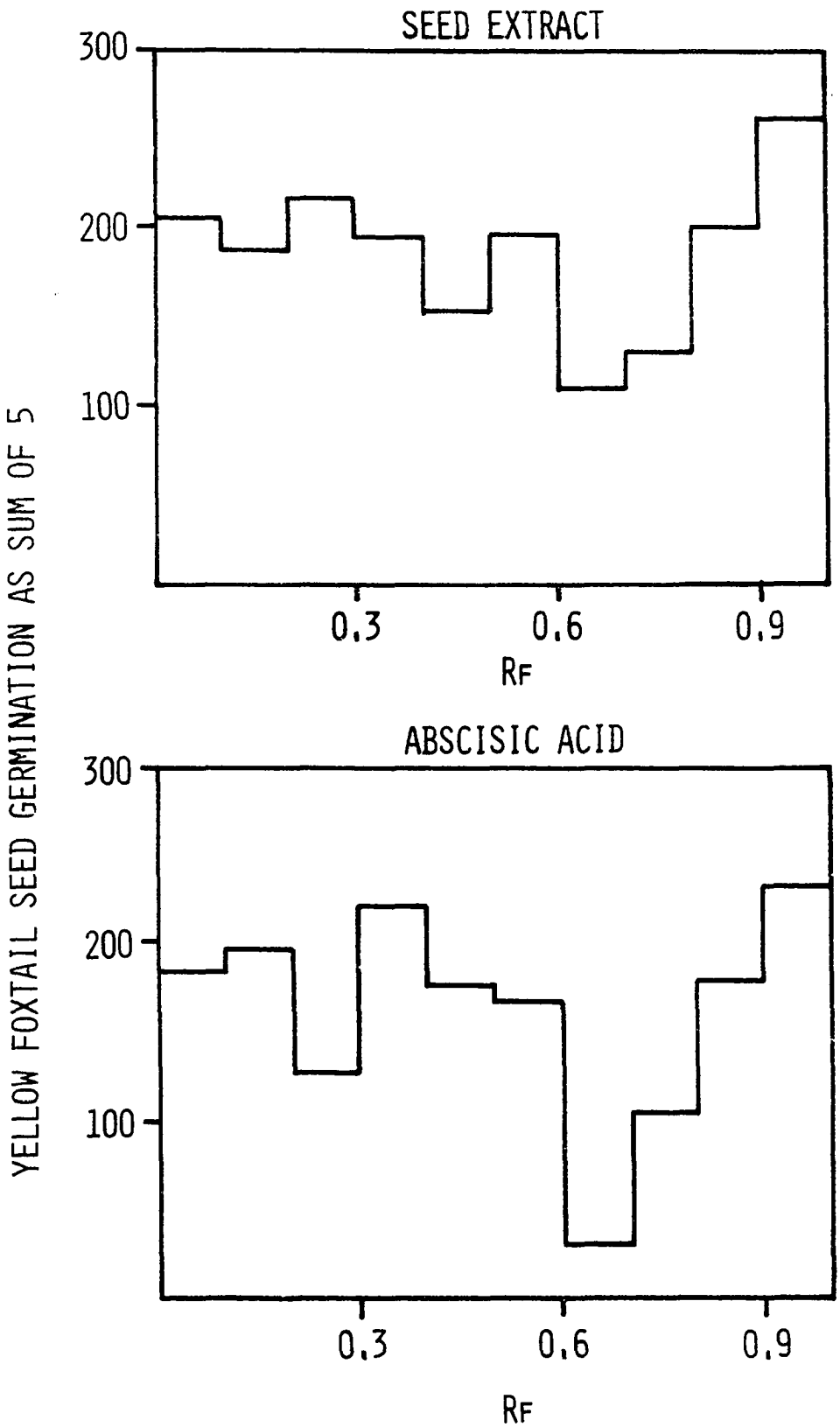
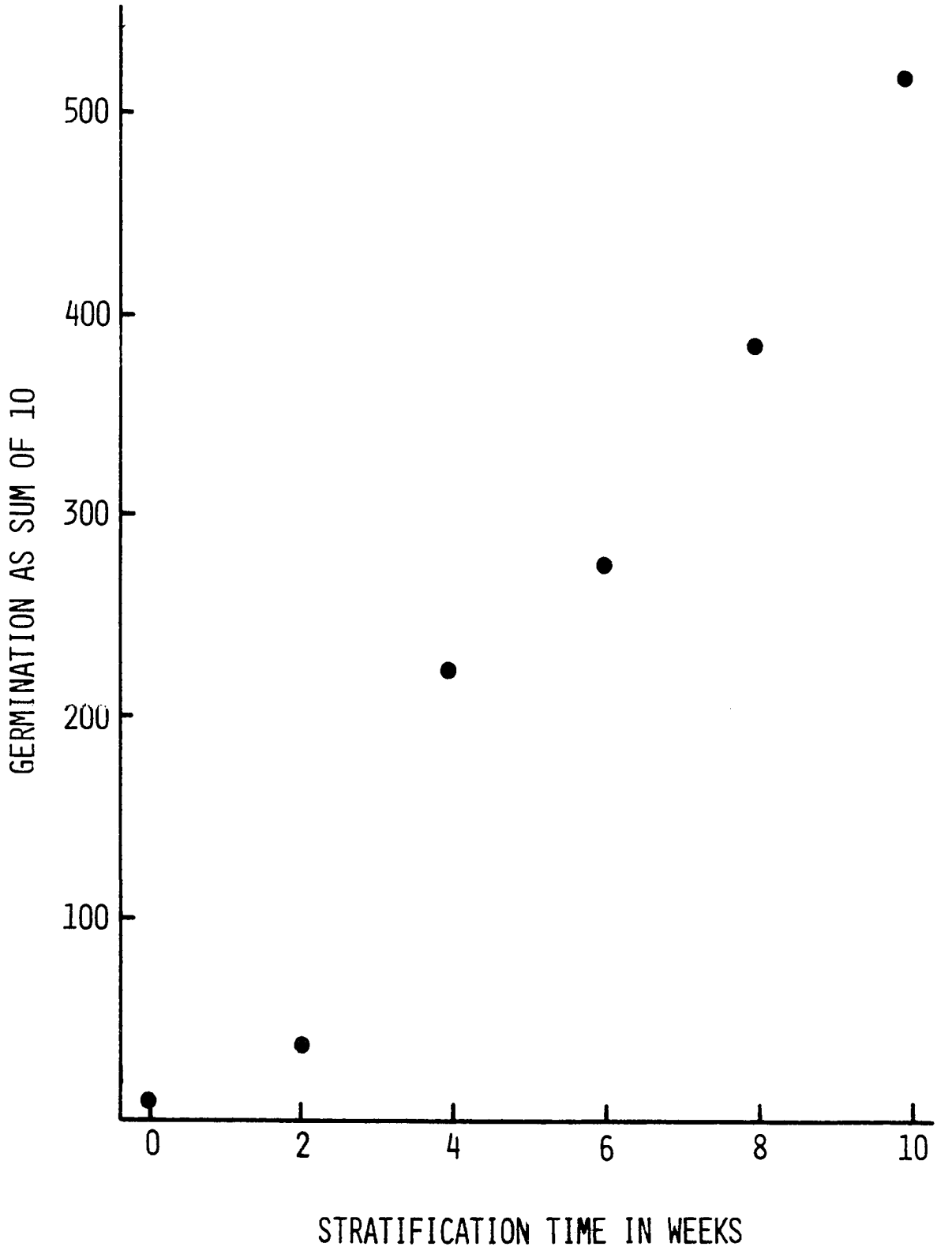

SEED EXTRACT
300
200
100
0.3
0.6
0.9
RF
ABSCISIC ACID
300
200
100
0.3
0.6
0.9
RF
YELLOW FOXTAIL SEED GERMINATION AS SUM OF 5

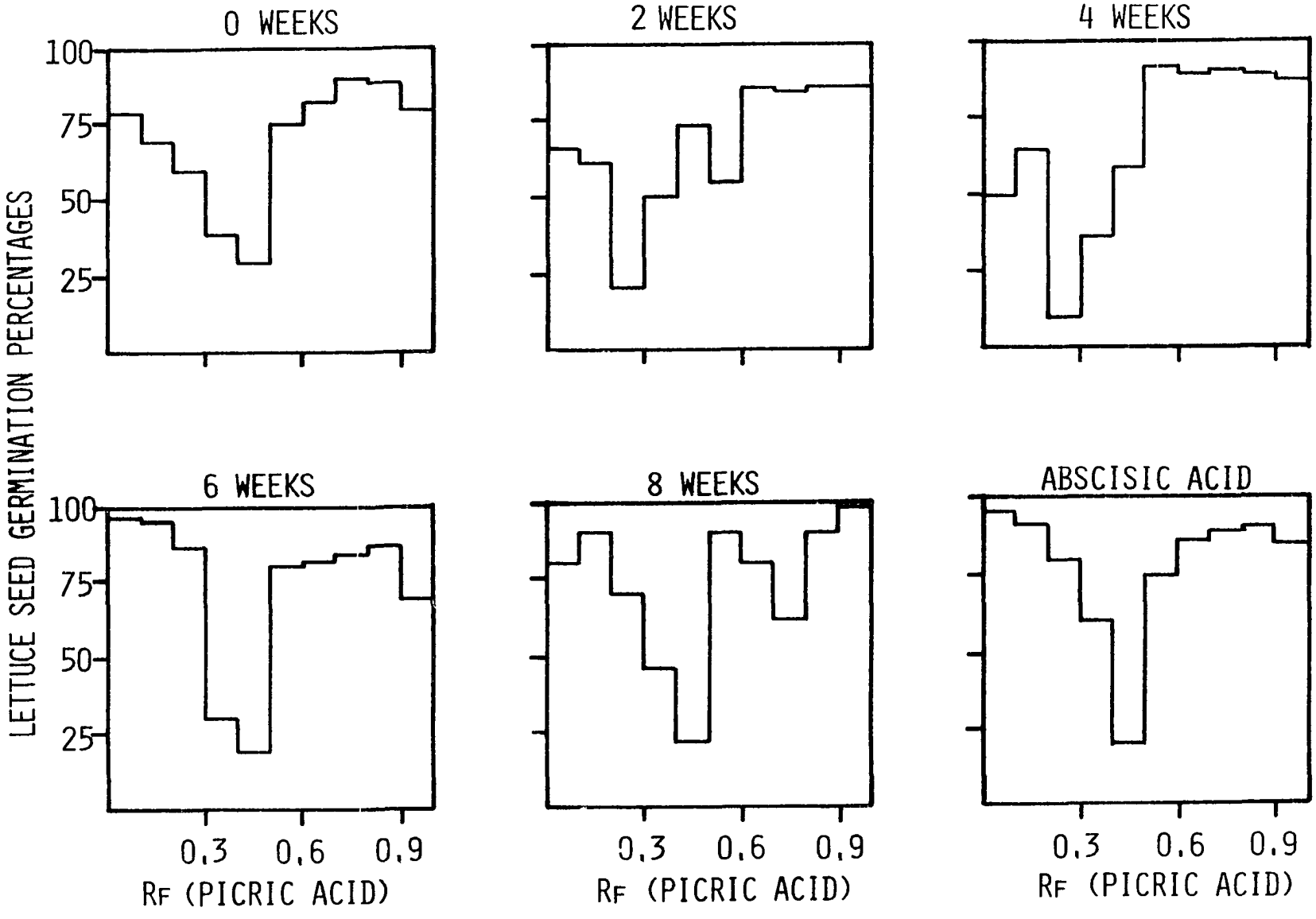
Figure 18. The effect of stratification on the germination potential of yellow foxtail seeds. The seeds were stratified under moist conditions at 5 C for various periods of time. Germination tests were conducted in a 15-25 C alternating temperature germinator, and each treatment consisted of four replicates of 100 seeds


500
400
300
200
100
GERMINATION AS SUM OF 10
0
2
4
6
8
10
STRATIFICATION TIME IN WEEKS

Because the germination potential can be greatly altered by a stratification treatment and because there is evidence that a germination inhibitor or inhibitors are involved in the control of seed germination, it was considered essential to examine the endogenous inhibitor level following various periods of stratification. Seed extracts were made from seeds stratified for 0, 2, 4, 6, 8, and 10 weeks. These extracts and the abscisic acid standard were subjected to paper electrophoresis and a lettuce seed germination bioassay. Figure 19 shows the comparisons of extract bioassays of seeds stratified for periods of time between 0 and 8 weeks. Inhibition was observed in each extract bioassay, and there was no evidence that the inhibitor level varied with stratification time.

These extracts represented 50 gms of seeds by fresh weight which is a relatively large extract for bioassay. Possibly all of the bioassays were saturated with inhibitor from the seed extracts, and differences between the various seed batches would be better revealed by smaller amounts of seed extract. To test this, a series of extracts from the two seed batches at the extremes of the stratification period (0 and 10 weeks) were compared. Eleven, 6, and 3 gm extracts were bioassayed for each of the two seed batches and compared. Paper chromatography was substituted for paper electrophoresis because less scattering of the endogenous

Figure 19. A comparison of the endogenous inhibitor levels of extracts from seeds which were stratified for 0, 2, 4, 6, and 8 weeks and an abscisic acid standard by the lettuce seed germination bioassay. The extracts and standard were separated by paper electrophoresis. The seed extracts were the equivalent of 50 gms of seed fresh weight, and 10 µg of abscisic acid was used as the standard


0 WEEKS
2 WEEKS
4 WEEKS
6 WEEKS
8 WEEKS
ABSCISIC ACID
LETTUCE SEED GERMINATION PERCENTAGES
100
75
50
25
0.3
0.6
0.9
RF (PICRIC ACID)

inhibitor occurred with that procedure. Figure 20 shows the comparisons of the bioassays between the dormant (non-stratified) and the nondormant (stratified 10 weeks) seeds. There were no clear cut differences between the two seed batches. Inhibition was evident from the 6 gm extracts, but not clearly detectable from the 3 gm extracts of both seed batches.

Determination of Endogenous Inhibitor Level

An approximate determination of the endogenous inhibitor level was made by comparison of a series of extracts, representing various amounts of starting material, and a series of abscisic acid standards. The seed extracts and abscisic acid standards were developed by paper chromatography and then subjected to bioassay. Figure 21 illustrates these comparisons. By these techniques the bioassay of the standard was sensitive to 0.2 µg of abscisic acid. Inhibition is evident on the chromatograph representing 0.2 µg of abscisic acid, but not clearly evident for 0.1 µg. Similarily for the seed extracts the chromatograph representing 6 gm of starting material shows inhibition, but little inhibition is noted for the 3 gm chromatograph. The best comparison between the two series is probably the 6 gm extract and the 0.2 µg standard. Based on that comparison the endogenous inhibitor level on a fresh weight basis is equivalent to approximately 30 µg/kg of abscisic acid.

Figure 20. A comparison of the endogenous inhibitor levels of extracts from non-dormant (10 weeks stratification) and dormant (nonstratified) seeds. Eleven, 6, and 3 gm extracts from both seed lots were compared by lettuce seed germination bioassay. Prior to the bioassay the extracts were separated by paper chromatography with butanol:1.5 N ammonium hydroxide (3:1) (organic phase) as the solvent

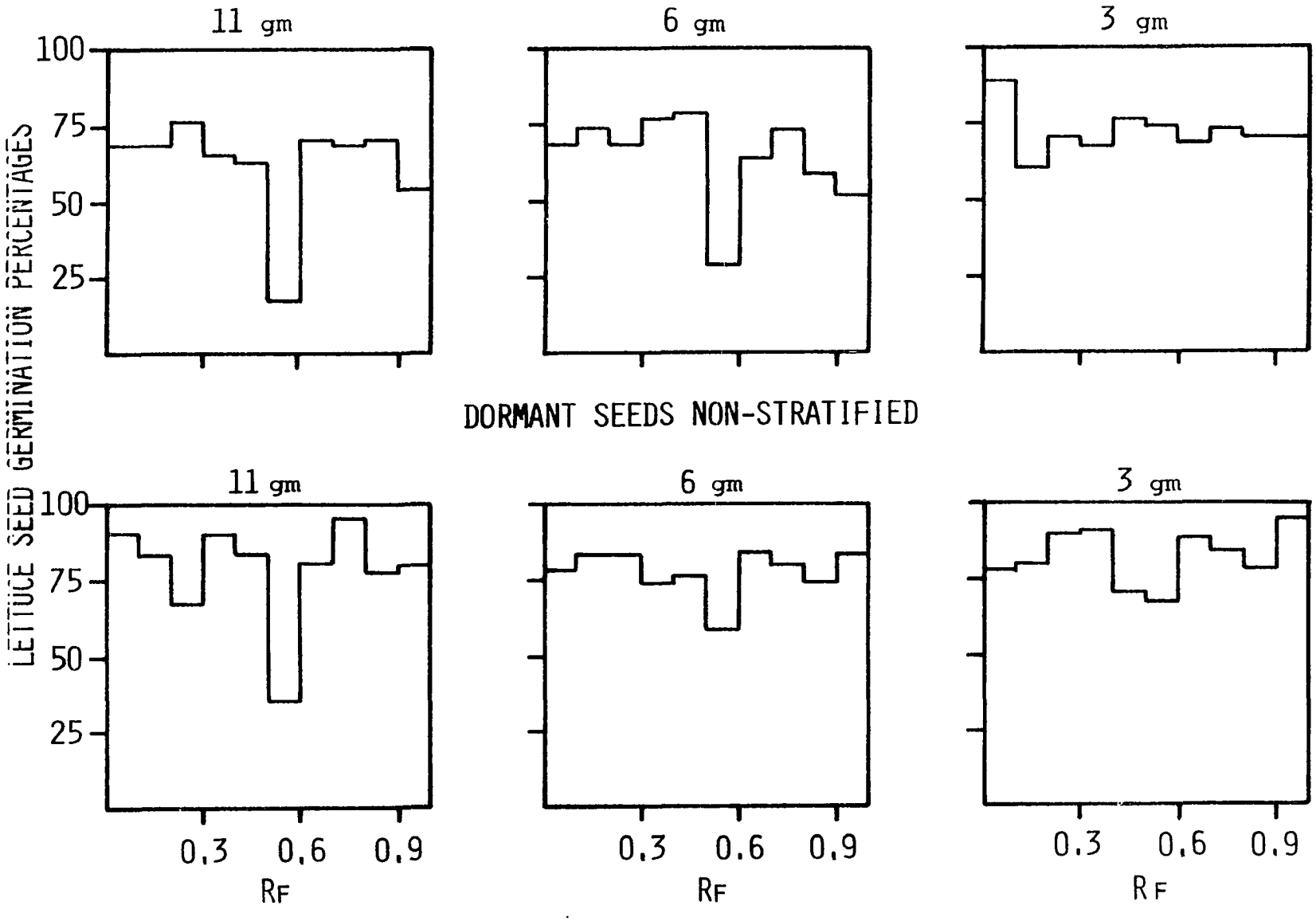
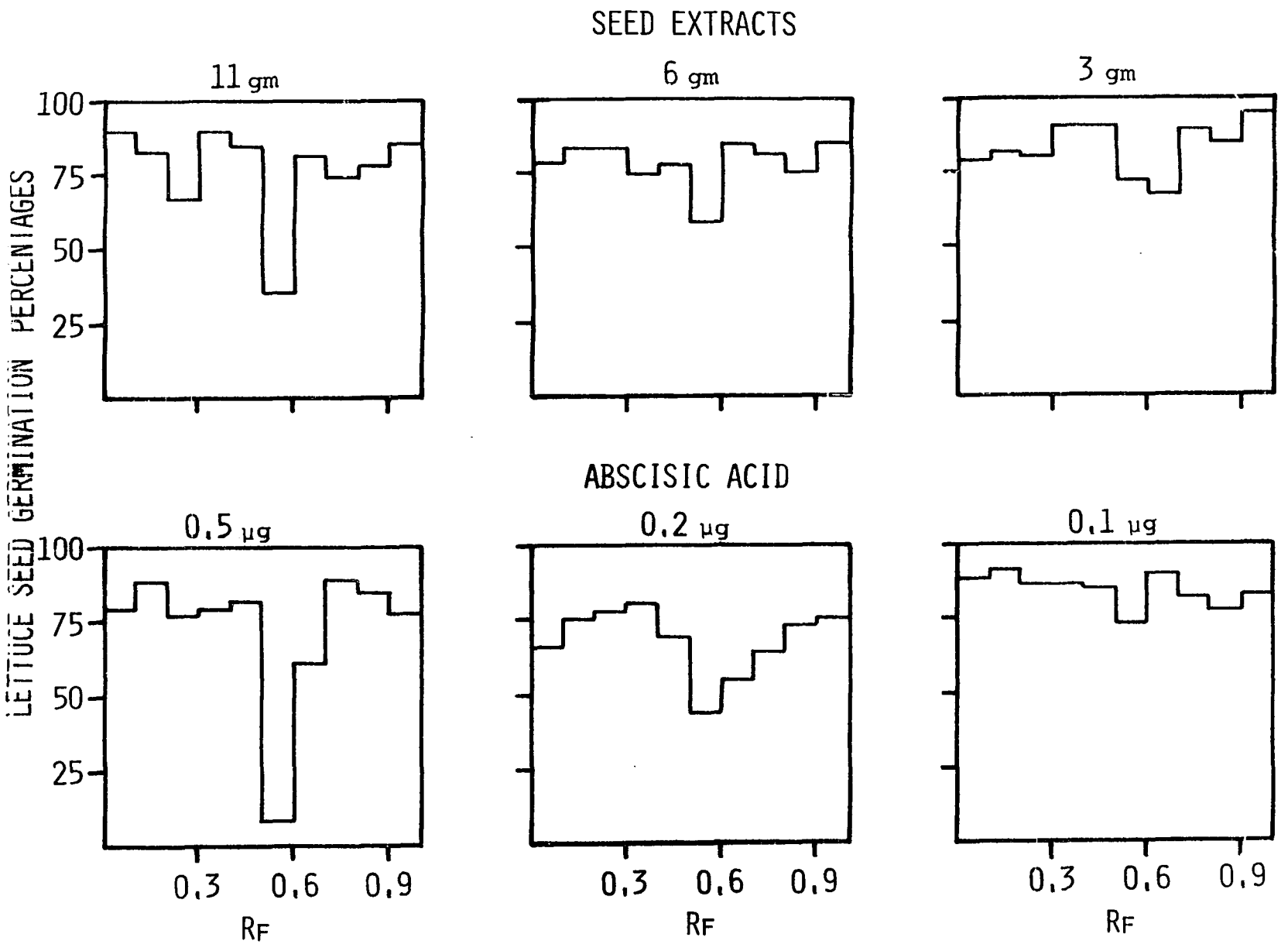

NON- DORMANT SEEDS STRATIFIED 10 WEEKS
11 gm
6 gm
3 gm
100
75
50
25
LETTUCE SEED GERMINATION PERCENTAGES
DORMANT SEEDS NON-STRATIFIED
11 gm
6 gm
3 gm
100
75
50
25
0.3
0.6
0.9
RF
0.3
0.6
0.9
RF
0.3
0.6
0.9
RF

Figure 21. A comparison of an inhibitor from a series of seed extracts (upper row) representing various amounts of starting material with a series of abscisic acid standards (lower row) by the lettuce seed germination bioassay. Prior to the bioassay the extracts and standards were subjected to separation by paper chromatography with butanol: 1.5 N ammonium hydroxide (3:1) (organic phase) as the solvent


SEED EXTRACTS
11 gm
6 gm
3 gm
ABSCISIC ACID
0.5 µg
0.2 µg
0.1 µg
LETTUCE SEED GERMINATION PERCENTAGES
100
75
50
25
0.3
0.6
0.9
RF

DISCUSSION

This investigation offers strong evidence that promoters and inhibitors are involved in the regulation of the germination and dormancy of yellow foxtail seeds. The germination of these seeds can be strongly influenced by treatment with exogenous hormones. Abscisic acid inhibits the germination of the seeds and two classes of hormones, the gibberellins and cytokinins, are effective promoters of germination. The inhibitory effects of abscisic acid can be partially reversed by the promoters. An endogenous inhibitor was extracted from yellow foxtail seed and found to be similar to abscisic acid in all systems examined. This inhibitor suppressed the germination of the species from which it was extracted.

Nieto-Hatem (1963) reported that there are two kinds of dormancy in yellow foxtail seeds--caryopses and hull. The results of this investigation support that contention. Included in this investigation were two dormant seed lots; one apparently in a state of hull dormancy, the other in caryopses dormancy. The seed lot exhibiting hull dormancy had nondormant caryopses as determined by caryopsis excision and germination tests. In addition, the dormancy of this seed lot was broken by acid treatment or by water leaching.

In contrast, the seed lot exhibiting caryopsis dormancy had dormant excised caryopses and did not respond

to acid treatment or water leaching. It is not clear if the two types of dormancy observed in the laboratory (caryopsis and hull) correspond with the two types of dormancy observed in the field (innate and induced). Hull dormancy which arose from storage in the laboratory under cool dry conditions may be an artificial form of dormancy.

The results obtained in this investigation concerning the effects of abscisic acid and gibberellin on the seed germination of yellow foxtail are similar to reports in the literature with other species. The inhibitory effect of abscisic acid was readily demonstrated in intact seeds; however, a positive germination response from gibberellin was obtained only after a disruptive treatment to the outer seed structures. Evidently the intact seed is impermeable to gibberellin. These findings agree with several reports in the literature which indicate that damage to the external structures of the seed by some treatment increases the effectiveness of gibberellin.

Excised caryopses were superior to intact seeds for the study of the effects of exogenous promoters. Unlike its effect on intact seeds gibberellin had a very strong effect on the germination of dormant caryopses. Several reports in the literature indicated that gibberellins other than gibberellic acid, mainly gibberellin A_4, are more effective in promoting germination. The findings of this investigation

that the combination product gibberellin A_4 plus gibberellin A_7 was more effective than gibberellic acid is consistent with those reports.

A promotive effect of cytokinins on dormant caryopses germination was demonstrated. Combinations of gibberellin and cytokinin resulted in an additive effect on the promotion of the germination of dormant caryopses. The results indicated that the effects of abscisic acid and benzyl-adenine were linear with concentration, and the effect of gibberelin was linear with the logarithm of the concentration.

In light of the proposal of Khan and Waters (1969) interesting results were obtained concerning the effect of promoters on the reversal of abscisic acid inhibition. They proposed that the function of cytokinins in seed germination is to specifically antagonize abscisic acid inhibition which allows gibberellin to function as a germination stimulus. According to the proposal gibberellins are ineffective in reversing abscisic acid inhibition.

The results of this investigation showed that the cytokinin benzyl-adenine was effective in reversing abscisic acid inhibition, and that gibberellic acid was ineffective. However, a combination of gibberellin and benzyl-adenine was more effective than the latter alone. These results are in accord with the Khan and Waters' proposal; however, the reversal of inhibition was not complete. Benzyl-adenine

alone or in combination with gibberellin reversed the inhibition of the coleoptile and primary leaf elongation, but at the other end of the embryonic axis the inhibition was not reversed. The inhibition which resulted in the failure of the radicle to penetrate the coleorhiza and elongate was not reversed by promoter treatment.

The proposal of Khan and Waters (1969) was based for a large part on the results obtained with promoter-inhibitor effects on lettuce seed germination as reported by Khan (1967), Khan (1968a), and Sankhla and Sankhla (1968a). These investigations were repeated in this laboratory, and it was found that the results from lettuce seed were very similar to those from yellow foxtail. As reported benzyl-adenine was effective in reversing the abscisic acid caused inhibition of lettuce seed germination, and gibberellic acid was ineffective in this respect. However, the germination resulting from the reversal of inhibition was abnormal. In normal lettuce seed germination, growth of the radicle occurs first which results in penetration of the seed coat. This is followed by elongation of the hypocotyl. In contrast, the germination that resulted from reversal of abscisic acid inhibition by a cytokinin, little or no radicle growth occurred, and elongation of the hypocotyl resulted in the disruption of the seed coat. Thus in the germination of yellow foxtail seeds as in lettuce seeds,

abscisic acid inhibition was reversed by benzyl-adenine at the shoot end of the embryonic axis but not at the root end.

Several explanations may account for the failure of the cytokinin in reversing the abscisic acid caused inhibition at the root end of the embryonic axis. The cytokinins are a class of hormones; complete reversal may require the use of a specific member. Possibly an adjustment of the levels or ratios of the hormones involved is required to obtain complete reversal. Failure to completely reverse the inhibition may simply reflect different reactions to exogenous and endogenous hormones. With the use of exogenous hormones the entire seed tissue is bathed in the hormone solution which may result in a more general response than from a specifically located endogenous hormone. On the other hand the exogenous hormone may not penetrate to all of the sites of action, and thus be partially ineffective. The reversal at the root end may require a compound other than a cytokinin.

The entire section of this investigation concerning the effects of exogenous promoters and inhibitors can be briefly summarized by classifying the promoter responses into two groups based on the effect on dormant caryopses, and the effect on nondormant caryopses inhibited by abscisic acid. This classification is tabulated in Table 4.

It is obvious from the table that the effect of the

Table 4. A summary of the effects of exogenous promoters and inhibitors on seed germination. The effect of germination promoters on the germination of dormant caryopses and nondormant caryopses inhibited by abscisic acid

Promoter(s)	Dormant caryopses	Nondormant caryopses inhibited by abscisic acid
gibberellin	promotes normal germination	no effect
benzyl-adenine	promotes normal germination	promotes abnormal germination[a]
gibberellin and benzyl-adenine	promotes normal germination additively	promotes abnormal germination[a] synergistically

[a]Abnormal germination refers to elongation of the coleoptile without a corresponding elongation of the radicle.

promoters is grossly different on the two groups of non-germinating caryopses. Gibberellin promotes the germination of dormant caryopses, but does not reverse inhibition from exogenous abscisic acid. Benzyl-adenine promotes normal germination of dormant caryopses, but abnormal germination of abscisic acid inhibited caryopses. The combination of the two promoters results in an additive effect on dormant caryopses, but a synergistic effect on the nondormant caryopses inhibited by abscisic acid.

Thus it appears that a dormant seed reacts differently to exogenous promoters than a nondormant seed inhibited by

exogenous abscisic acid. These differences may be interpreted to indicate that abscisic acid is not the natural cause of dormancy in yellow foxtail seeds. However, an alternate explanation would be that the specific effect resulting from an endogenous inhibitor and the general effect resulting from an exogenously applied inhibitor could account for the observed differences.

The results from the first group of nongerminating caryopses, the dormant caryopses, are consistent with the Khan and Waters' proposal if it is assumed that the dormancy is caused by an endogenous inhibitor and that small amounts of endogenous gibberellin and cytokinin are present but in suboptimal levels. Thus gibberellin would promote germination by providing the germination stimulus, and cytokinin by reversing the inhibition of the endogenous inhibitor. A combination of the two would be most effective. The results from the second group of nongerminating caryopses, the nondormant caryopses inhibited by abscisic acid, has been discussed with regard to the Khan and Waters' proposal.

The results of this investigation concerning the effects of exogenous promoters and inhibitors are generally consistent with the proposal of Khan and Waters. The primary inconsistency results from the failure of the cytokinin to reverse the abscisic acid inhibition at the root end of the embryonic axis. This investigation indicates that the proposal may

serve as a good working hypothesis for the study of the regulatory mechanism of seed germination and dormancy.

An endogenous inhibitor was extracted from yellow foxtail seeds and found to be similar to abscisic acid in all systems examined. The R_f value of the endogenous inhibitor was found to be very similar to the R_f value of abscisic acid in each of the six paper chromatography solvent systems employed. The migration of the inhibitor was very similar to the migration of abscisic acid in each of three paper electrophoresis runs in which the pH of the buffer was varied. This additional comparison by electrophoresis adds substantially to the evidence that the endogenous inhibitor is identical to abscisic acid. The two methods separate on the basis of different characteristics; paper chromatography is based on partition between two liquid phases, paper electrophoresis is based on the electrostatic charge of the molecule at a particular pH. Three bioassays were employed to examine the effects of the endogenous inhibitor relative to abscisic acid. Both inhibited lettuce seed germination, yellow foxtail seed germination, and the gibberellin-induced production of α-amylase by barley endosperms. These results represent strong but not conclusive evidence that the isolated inhibitor is abscisic acid.

No difference was detected in the inhibitor level between dormant and nondormant (stratified) seeds. However, it is

not clear if the endogenous inhibitor level does not decline during the termination of dormancy or if the decline is so small that it was not detectable by the methods employed. Several investigators Martin et al. (1969), Rudnicki (1969), and Lipe and Crane (1966) reported large declines in the inhibitor content of the seeds of three woody species during dormancy release. Contrary to the results of this investigation, they could find no inhibitor present in nondormant seeds. However, Sondlheimer et al. (1968) reported only a 37 percent decline of endogenous inhibitor in the pericarp and a 68 percent decline in the seed during stratification of ash seeds. The chromatography and bioassay method of detecting inhibitors employed in this investigation would detect large changes in the inhibitor level, but would not detect a subtle change. If the critical decline in inhibitor level for the release of dormancy was only at a specific site, the overall level of inhibitor in the seed may change very little.

It was determined further that the approximate level of inhibitor extracted form the seeds was equivalent to 30 µg of abscisic acid per kg of fresh weight. This is in close agreement with published reports. Rudnicki (1969) reported 40 µg/kg of abscisic acid extracted from apple seeds. Milborrow (1967) reported 34 µg/kg for avocado seed, 46 µg/kg for linden pericarp, and 83 µg/kg for corn fruit as

determined by standard methods. Three to four times greater values were obtained with the quantitative racemate dilution method. Evidently losses which occurred during the extraction and purification procedures account for the lower amount obtained by the standard method. The racemate dilution technique employs the use of an internal standard which compensates for these losses.

There is considerable evidence that inhibitors and promoters are involved in the germination and dormancy of yellow foxtail seeds, but it remains unclear exactly what changes in these compounds take place during the transformation from a dormant to a nondormant seed. There are two major possibilities; the inhibitor is deactivated by some means or a promoter is activated which overcomes the effect of the inhibitor. There is evidence in the literature supporting both contentions. Martin et al. (1969), Rudnicki (1969), and Lipe and Crane (1966) have shown large decreases in inhibitor level in the transformation from a dormant to a nondormant seed in several woody species. Bradbeer (1968) showed that stratification which releases dormancy in hazel seeds increased the potential of the seeds to produce gibberellin. Khan (1967a), Khan (1968), and Sankhla and Sankhla (1968a) demonstrated that cytokinins were able to reverse the effect of germination inhibitors.

In this investigation there was no evidence that the

endogenous inhibitor isolated from the seed extract decreased in amount during the transformation from a dormant to a nondormant seed. However, the possibility remains that the situation for the inhibitor may be similar to that for gibberellin in hazel seed as demonstrated by Bradbeer (1968). The potential to deactivate the endogenous inhibitor may be developed during stratification but the actual deactivation occurs during the subsequent germination process. This possibility was not examined.

However, this investigation does offer strong evidence that a promoter (cytokinin) was able to reverse the effects of an inhibitor. This reversal was striking but not complete. The reversal of inhibition occurred only at the shoot end of the embryonic axis.

This investigation offers evidence, although not conclusive, that the inhibitor level is not decreased during the transformation from a dormant seed to a nondormant one; and that promoters, possibly activated or the potential activated during stratification, can reverse the effect of the inhibitor. Thus dormancy could be terminated without the elimination of the endogenous inhibitor.

SUMMARY

1. This study was an attempt to characterize the regulatory mechanism of seed germination in yellow foxtail and was based on the proposal that germination promoters and inhibitors are involved in the control of these processes.
2. It was found that high voltage paper electrophoresis was a very effective means of separating the two plant hormones abscisic acid and gibberellic acid.
3. Evidently these seeds exhibit two kinds of dormancy--hull and caryopsis. Hull dormancy can be terminated by removal of the lemma and palea (hull), acid treatment, or water leaching. These treatments do not affect caryopsis dormancy which can be terminated by stratification.
4. No differences were found in the rate of water uptake between dormant and nondormant seeds.
5. Experiments with exogenous test solutions showed that abscisic acid inhibited seed germination and that the gibberellins and cytokinins promoted the germination of this species. The effect of the promoters was much more readily demonstrated with excised caryopses than with intact seeds.
6. Benzyl-adenine reversed abscisic acid inhibition of germination; gibberellin did not, but the two promoters in combination were more effective than the cytokinin alone. The reversal occurred only at one end of the

embryonic axis--the shoot end.

7. The proposed gibberellin synthesis inhibitors CCC and AMO 1618 had no effect on the germination of yellow foxtail seeds.
8. An endogenous inhibitor was extracted from yellow foxtail seeds and was found to be similar to abscisic acid in all systems examined. The extracted inhibitor was compared to abscisic acid in six paper chromatography solvent systems, three paper electrophoresis runs at different pH's, and three different bioassays.
9. No difference was detected in the level of endogenous inhibitor between dormant and nondormant (stratified) seeds.
10. It was determined that the approximate level of inhibitor extracted from the seeds was equivalent to 30 µg of abscisic acid per kg of fresh weight.

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ACKNOWLEDGMENTS

The author is indebted to Dr. D. W. Staniforth for suggesting the problem, for his helpful direction during the course of this study and in the preparation of the manuscript. A special note of thanks to my wife, Hazel, for her assistance and encouragement during the course of this investigation.